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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN THORACIC SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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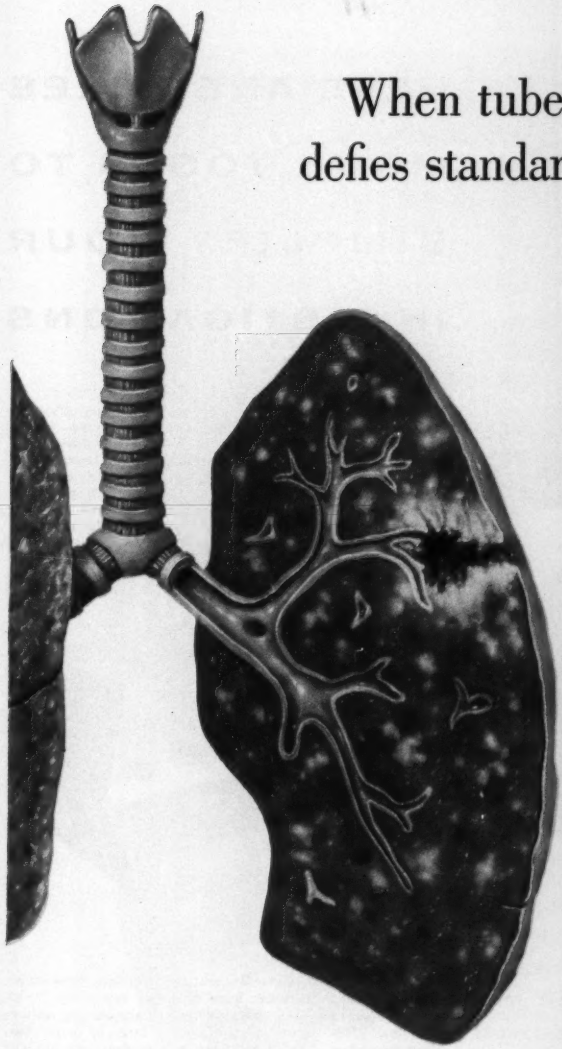

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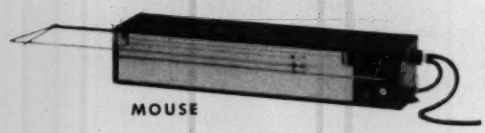

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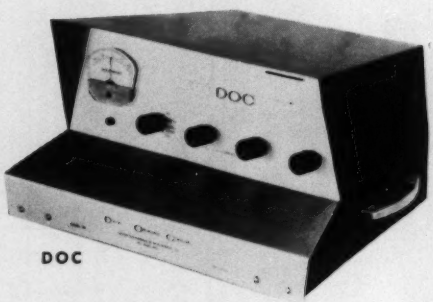

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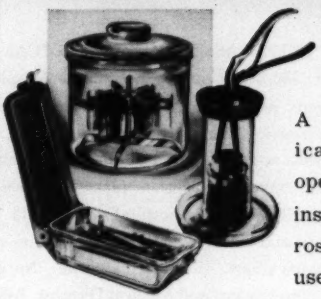

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The Editor wishes to take this opportunity to express his appreciation to Dr. William R. Barclay, Dr. Aaron D. Chaves, Dr. Leon H. Schmidt, and Dr. Robert L. Yeager for their high quality of performance and diligent service as members of the Editorial Board.

New members of the Board as of July 1, 1960, are Dr. Dieter Koch-Weser, Dr. Cynthia Pierce, and Dr. Arthur Robins, who have accepted three-year appointments.

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CLINICAL USEFULNESS OF RIBOFLAVIN-TAGGED ISONIAZID FOR SELF-MEDICATION IN TUBERCULOUS PATIENTS[1, 2]

KURT W. DEUSCHLE, CLARENCE JORDAHL, AND GLADYS L. HOBBY

(Received for publication February 4, 1960)

Introduction

The clinician who places a patient on self-medication cannot predict whether or not the patient will follow the regimen as prescribed. If the patient has an acute health problem, such as severe diabetes, the subsequent course of his disease may soon reveal the regularity with which he follows the prescribed treatment program. However, if the patient has a chronic illness such as tuberculosis, the failure to take medication regularly may not be so readily discerned. In this situation, the physician must rely on his "clinical intuition" to appraise what is happening. The range of diseases treated on an ambulatory self-medication basis is constantly increasing; and there is a need, therefore, for a precise, objective method of evaluating the regularity with which patients take drugs in the absence of direct supervision.

A method whereby the patients on drug self-administration may be characterized as "regular" or "irregular" could be established if the prescribed drug could be readily identified in the urine. Most therapeutic drugs, however, are not excreted in the urine in a form which can be easily detected by chemical or biologic methods in the average hospital laboratory. Isoniazid, in particular, the major component of anti-tuberculous chemotherapy, can be detected only with difficulty.

It is especially important in domiciliary programs, or in programs designed to evaluate drug regimens which include other chemotherapeutic agents administered simultaneously with isoniazid, to know the regularity with which these drugs are taken. Several techniques for the detection of isoniazid have been described for field use (1–3); yet none have been wholly satisfactory.

Data were presented in a previous communication by the present writers (4) to show that isoniazid may be tagged with riboflavin, a compound easily detected upon excretion in urine. The suggestion was made that this compound might be used to "tag" not only isoniazid, but other selected therapeutic agents. Attention was called to the fact, however, that the presence of riboflavin in various concentrations in many foods and in supplementary vitamin preparations, and its presence in normal urine, might interfere with its use in this manner.

In the present report, data will be presented which again illustrate the ease with which riboflavin may be detected in the urine of patients receiving this substance orally. In addition, evidence will be presented to support the belief that this drug may be used as a means of detecting those persons who accept isoniazid therapy. The data were derived from tuberculous patients treated under direct supervision in a hospital ward and from tuberculous patients receiving home-care.

Method

Scored isoniazid tablets containing 50 mg. of active drug were matched in size and color to tablets containing 50 mg. of isoniazid and 8 mg. of

[1] From the Department of Public Health and Preventive Medicine and the Department of Medicine, the New York Hospital-Cornell Medical Center, New York, New York; and the Special Research Laboratory, U. S. Veterans Administration Hospital, East Orange, New Jersey.

[2] This work has had the generous support of the Navajo Tribe; the U. S. Public Health Service, Division of Indian Health; the National Institutes of Health, U. S. Public Health Service; and Chas. Pfizer and Co., Inc.

riboflavin.[3] The ratio of 50 mg. of isoniazid to 8 mg. of riboflavin was selected on the basis of past experience, indicating that this is the least amount of riboflavin that will allow easy detection of amounts of the drug in excess of that frequently present in normal urine.

Isoniazid alone was administered as "placebo" medication to some patients and at some time periods, while isoniazid with riboflavin added was employed as the drug under test. Inasmuch as the "placebo" medication contained isoniazid, it was not possible to distinguish this medication from riboflavin-tagged isoniazid by taste. In all instances, isoniazid was administered orally in dosages equivalent to 5 or 10 mg. of drug per kg. of body weight. In those patients receiving riboflavin in combination with isoniazid, therefore, the amount of riboflavin administered was always equivalent to 0.8 or 1.6 mg. per kg. of body weight.

In a portion of the study, vitamin capsules (multiple-formula vitamins), each containing 3 mg. of riboflavin, were also administered. In this manner it was hoped to determine the extent to which vitamin therapy might interfere with the detection of those riboflavin concentrations produced by the administration of riboflavin-tagged isoniazid.

The technique employed throughout the study for determination of the concentration of riboflavin in urine has been described elsewhere (4). Concentrations of riboflavin equal to or less than 2.5 γ per ml. of urine were accepted as *certain* evidence that the patient had not received riboflavin with isoniazid, while concentrations in excess of 2.4 but less than 3.5 γ per ml. were accepted as *probable* evidence that riboflavin-tagged isoniazid had not been ingested (4).

Patients: Volunteer patients on the chest service (ward) of The New York Hospital, domiciliary patients from the same hospital, and patients from the Many Farms Clinic[4] on the Navajo Reservation were employed throughout the study.

Observations

A. *Riboflavin Excretion in Two Ward Patients*

Two hospitalized patients on the chest service of The New York Hospital were used in the initial phase of this study to evaluate riboflavin intake and output.

Urine specimens were collected from each of the 2 patients daily for nine days. On the second day of the experiment, patient *No. 1* received one vitamin capsule containing 3 mg. of riboflavin, while patient *No. 2* was given two such capsules. The riboflavin dosage thus was equivalent to 0.07 and 0.1 mg., respectively, per kg. of body weight. On the seventh day of the experiment, riboflavin was administered in the form of riboflavin-tagged isoniazid in dosages equivalent to 1.6 and 0.8 mg., respectively, per kg. of body weight. Both patients received regular hospital diets, without vitamin supplementation, on the other days of the experiment. Medication was given under direct supervision at all times.

The output of riboflavin by these 2 patients during the course of this experiment is illustrated in figure 1. Patient *No. 1* excreted between 0.8 and 1.9 mg. (average 1.35 mg.) of riboflavin per twenty-four-hour period during a six-day observation period on a standard hospital diet, 2.8 mg. on a single day after administration of a riboflavin-containing vitamin capsule (dosage equivalent to 0.07 mg. riboflavin per kg. of body weight), and 8.65 mg. after administration of riboflavin-tagged isoniazid in an isoniazid dosage equivalent to 10 mg. per kg. of body weight, i.e., riboflavin equivalent to 1.6 mg. per kg. of body weight. Patient *No. 2* similarly excreted between 0.7 and 1.31 mg. (average 1.11 mg.) of riboflavin per twenty-four-hour period during a four-day period of observation on the routine hospital diet; 5.6 mg. after administration of two riboflavin-containing capsules (dosage equivalent to 0.1 mg. of riboflavin per kg. of body weight); and 14.7 mg. after administration of riboflavin-tagged isoniazid in a dosage equivalent to 5 mg. of isoniazid per kg. of body weight, i.e., riboflavin equivalent to 0.8 mg. per kg. of body weight.

The differences between the concentrations of riboflavin in normal urine, in the urine following vitamin supplementation, and in the urine following administration of riboflavin with isoniazid thus were readily apparent.

The concentrations of riboflavin in the urine of these 2 patients at 8 a.m. and 8 p.m. on each day during the course of the experiment are illustrated in figure 2. In each instance, medication, when given, was administered between 7 a.m. and 8 a.m.; the 8 p.m. specimen thus represented a twelve-hour post-therapy specimen. Of interest is the fact that the concentration of riboflavin in these 2 patients was invariably higher in the morning than in the evening, *except* when riboflavin had been administered either in the form of a vitamin capsule or in combination with isoniazid.

[3] The writers are indebted to Mr. E. Blase and Dr. R. Feeney of Chas. Pfizer & Co., Inc., Brooklyn, New York, for the preparation of the isoniazid and isoniazid-riboflavin tablets. The size, shape, and color of these two types of tablet were identical.

[4] The Many Farms Clinic is the central medical facility of the Cornell-Navajo Field Health Research Project, located on the Navajo Reservation in Arizona.

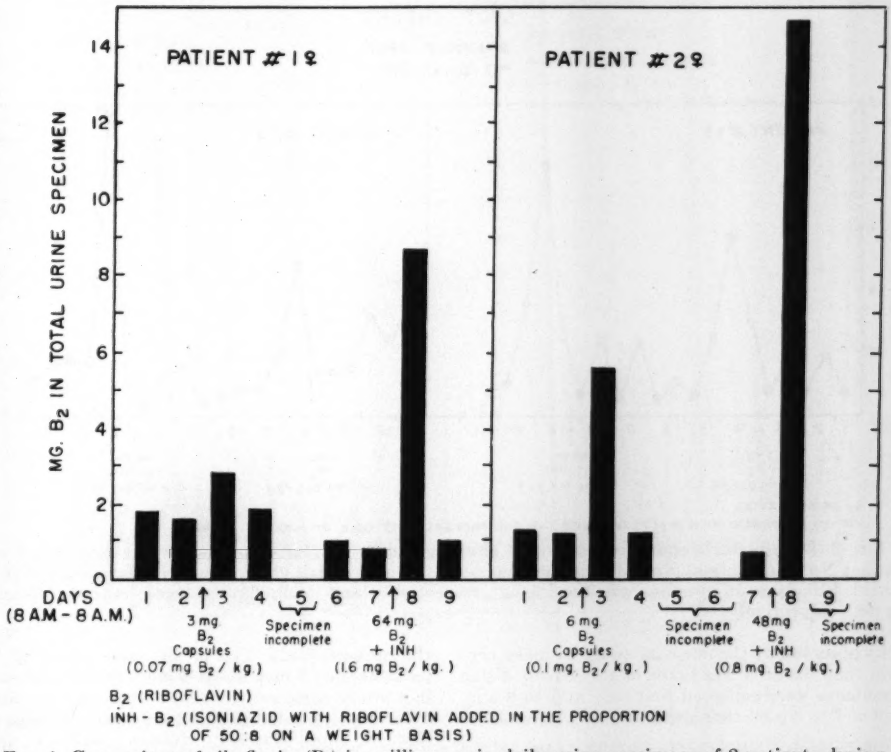
PATIENT # 1 ♀
PATIENT # 2 ♀
MG. B_2 IN TOTAL URINE SPECIMEN
0
2
4
6
8
10
12
14
DAYS (8 AM - 8 A.M.)
1 2 3 4 5 6 7 8 9
3 mg. B_2 Capsules (0.07 mg. B_2 / kg.)
Specimen incomplete
64 mg. B_2 + INH (1.6 mg. B_2 / kg.)
6 mg. B_2 Capsules (0.1 mg. B_2 / kg.)
Specimen incomplete
48 mg. B_2 + INH (0.8 mg. B_2 / kg.)
Specimen incomplete
B_2 (RIBOFLAVIN)
INH - B_2 (ISONIAZID WITH RIBOFLAVIN ADDED IN THE PROPORTION OF 50:8 ON A WEIGHT BASIS)

FIG. 1. Comparison of riboflavin (B_2) in milligrams in daily urine specimens of 2 patients during a nine-day observation period on a standard hospital diet following administration of a vitamin-containing riboflavin and the tablets of riboflavin-tagged isoniazid.

The volume of urine excreted per eight-hour period (12 midnight to 8. am., 8 a.m. to 4 p.m., and 4 p.m. to 12 midnight), the number of micrograms of riboflavin per milliliter from a urine sample during an eight-hour collection period, and the total number of milligrams of riboflavin per eight-hour aliquot are compared in figure 3. From the data presented it is apparent that the greatest quantity of riboflavin was excreted during the first eight-hour period following drug administration (8 a.m. to 4 p.m.). There was no evidence that riboflavin was stored within the body, nor was there any indication that the amount excreted was related to the volume of urine eliminated.

The total amount of riboflavin excreted per eight-hour period, in contrast to the concentration per milliliter of urine sampled in an eight-hour collection period is illustrated in figure 4. It is again evident that the riboflavin concentrations were highest during the 8 a.m. to 4 p.m. period in each instance.

It may be seen (figures 3, 4) that the concentration of riboflavin in whole eight-hour urine specimen parallels that found in the urine samples obtained sometime during each eight-hour collection period.

B. *Isoniazid with and without Riboflavin Added in Ward Patients*

Patients *No. 1* and *No. 2* and, in addition, 10 other hospitalized patients were employed in a subsequent evaluation of the excretion of riboflavin. Seven men and 5 women were included in the group under study. All patients were observed over a thirteen-day period. Isoniazid was administered as "placebo" on the first day, or on the first and second days of the experiment; thereafter isoniazid with riboflavin added was given to each patient daily for a period of seven to eight days, after which isoniazid alone was again administered as "placebo." In all instances, patients were care-

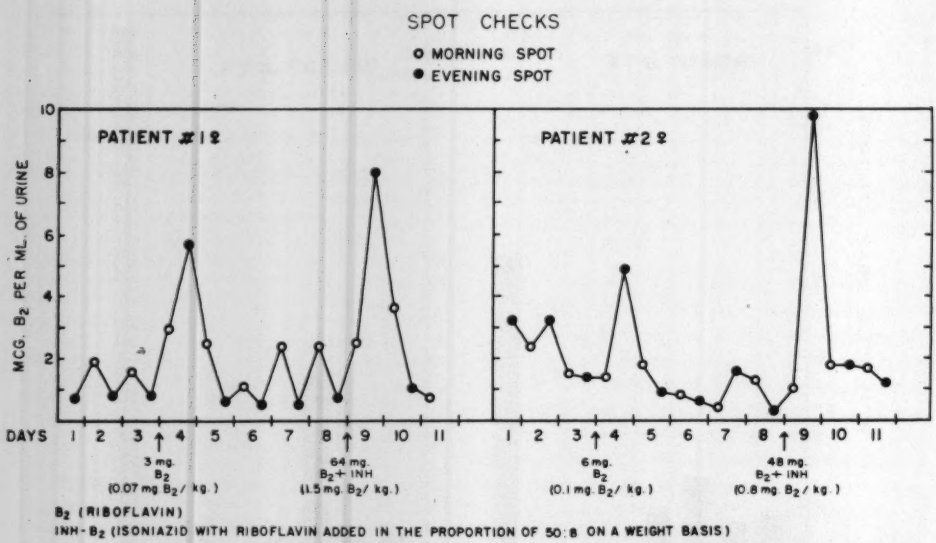
SPOT CHECKS
O MORNING SPOT
● EVENING SPOT
PATIENT # 1 ♀
PATIENT # 2 ♀
MCG. B2 PER ML. OF URINE
DAYS
3 mg. B2 (0.07 mg B2/ kg.)
64 mg. B2+ INH (1.5 mg. B2/ kg.)
6 mg. B2 (0.1 mg. B2/ kg.)
48 mg. B2+ INH (0.8 mg. B2/ kg.)
B2 (RIBOFLAVIN)
INH-B2 (ISONIAZID WITH RIBOFLAVIN ADDED IN THE PROPORTION OF 50:8 ON A WEIGHT BASIS)

FIG. 2. The riboflavin concentration in the morning and evening urine specimens was determined on patient No. 1 and patient No. 2 during the same period of study with vitamin and riboflavin-tagged isoniazid. Riboflavin in vitamins was 3 and 6 mg., respectively, and in riboflavin-tagged isoniazid, 64 and 48 mg., respectively.

fully observed by the nurse in charge to make certain that the drug was taken as prescribed. Urine specimens were collected routinely at 7 to 8 a.m. and at 7 to 8 p.m. each day.

The results obtained in this experiment are illustrated in figure 5. The concentrations of riboflavin in urine specimens collected twelve to fifteen hours after ingestion of isoniazid with riboflavin added were invariably well in excess of the normal range for each patient. In all instances, however, the concentrations of riboflavin returned to normal within forty-eight hours after the last dose of the riboflavin-containing drug, i.e., isoniazid with riboflavin added, had been administered.

It is of interest to note that patient *No. 5* showed a baseline riboflavin concentration equivalent to 8 γ per ml. of urine. Subsequent check of the patient's diet showed that he had received two vitamin capsules containing 6 mg. of riboflavin on the day that the baseline specimen was collected.

C. *Isoniazid with Riboflavin Added versus Riboflavin in Supplementary Vitamin Preparation; Ward Patients*

A period of five weeks was allowed to elapse between the above-described experiment (B) and the present study. Ten of the same group of 12 patients, i.e., 7 men and 3 women, were employed in a study designed to evaluate the relative concentrations of riboflavin obtained on administration of supplementary vitamin capsules and isoniazid with riboflavin added. Drugs were administered under direct supervision, and urine specimens were collected three times daily (7 to 8 a.m., 12 noon to 1 p.m., and 7 to 8 p.m.). The concentration of riboflavin in each specimen was then determined by the method described.

The results in this experiment are illustrated in figure 6. As might be expected, the concentration of riboflavin was highest in all instances in those specimens collected within four to six hours after the administration of either supplementary vitamin capsules or isoniazid with riboflavin added. The marked difference, however, between the concentrations obtained after the administration of the vitamin preparation and those obtained after the administration of isoniazid with riboflavin added is readily apparent from the results presented. With few exceptions, the riboflavin concentrations excreted in the urine following the administration of the vitamin capsules were less than 3.5 γ per ml. On the other hand, urine riboflavin concentrations following the isoniazid-riboflavin medica-

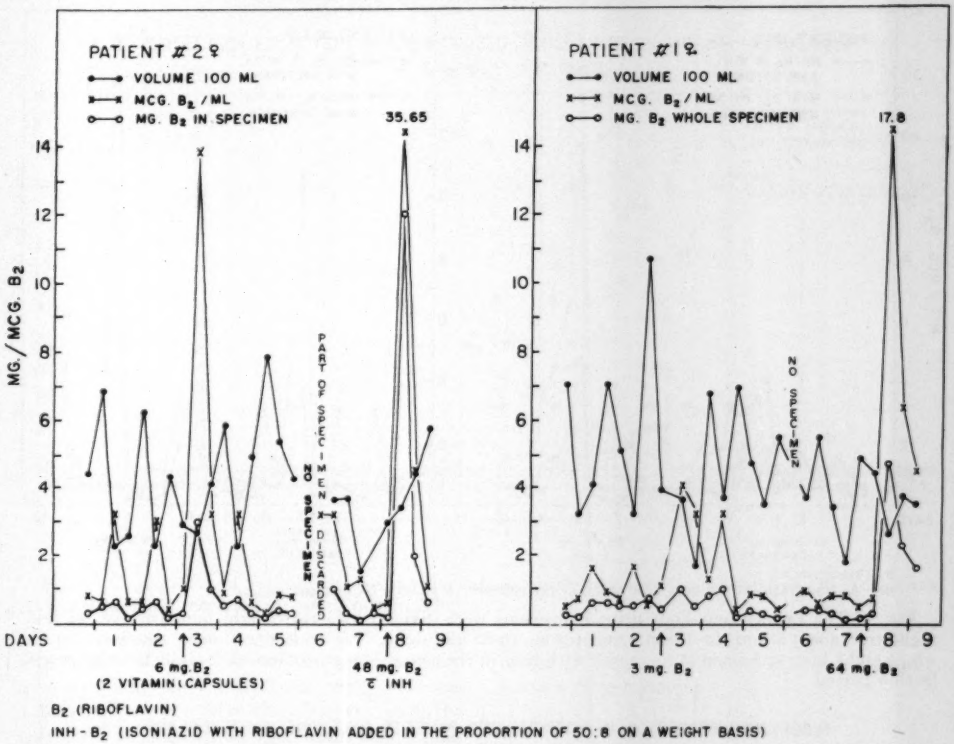

FIG. 3. The twenty-four-hour urine collections were divided into three eight-hour periods: 12 midnight to 8 a.m.; 8 a.m. to 4 p.m.; and 4 p.m. to 12 midnight. The volume of urine and concentration of riboflavin in micrograms per milliliter and for the total specimen are charted for each collection day.

tion were usually well above this value, ranging up to 100 γ per ml.

D. *In Domiciliary Patients at The New York Hospital and The Many Farms Clinic*

In order to determine the practicability of using riboflavin as a means of "tagging" isoniazid in patients treated on an outpatient or unsupervised basis, 9 New York Hospital clinic patients and 15 Navajo patients attending the Many Farms Clinic were employed in a subsequent study. All patients were given isoniazid with riboflavin added and instructions as to when the drug should be taken. Urine specimens were collected at the time of routine clinic visits or when a member of the clinic staff made a home visit. At no time were patients informed of the purpose of collecting the urine specimens.

The results in New York domiciliary patients are illustrated in figure 7*A*, *B*. The period of observation in these 9 patients extended over two to six months. The urine specimens were collected on unscheduled home or routine clinic visits.

In patient *No. 1* (L. F.) the riboflavin concentrations in the urine were consistently more than 2.5 γ per ml. except on two occasions. Subsequently it was learned that this patient had exhausted her supply of riboflavin-tagged isoniazid and had taken isoniazid without added riboflavin on these days. When the riboflavin-tagged isoniazid was resumed, high concentrations of riboflavin again appeared in the urine. There can be little doubt that this subject exemplifies a consistently cooperative patient who follows the prescribed regimen of daily drug self-administration.

Moreover, the riboflavin patterns in urine specimens from patients *No. 2* through *No. 7* indicated a high degree of regularity in self-

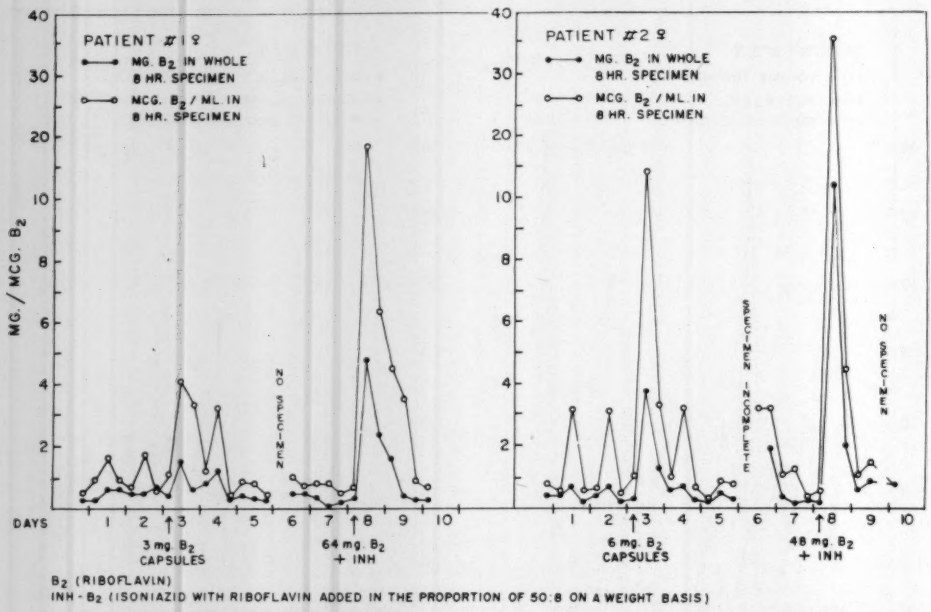
PATIENT #1 ♀
MG. B_2 IN WHOLE 8 HR. SPECIMEN
MCG. B_2 / ML. IN 8 HR. SPECIMEN
PATIENT #2 ♀
MG. B_2 IN WHOLE 8 HR. SPECIMEN
MCG. B_2 / ML. IN 8 HR. SPECIMEN
MG. / MCG. B_2
DAYS
NO SPECIMEN
3 mg. B_2 CAPSULES
64 mg. B_2 + INH
6 mg. B_2 CAPSULES
48 mg. B_2 + INH
B_2 (RIBOFLAVIN)
INH - B_2 (ISONIAZID WITH RIBOFLAVIN ADDED IN THE PROPORTION OF 50:8 ON A WEIGHT BASIS)

FIG. 4. The twenty-four-hour urine collections were divided into three eight-hour periods: 12 midnight to 8 a.m.; 8 a.m. to 4 p.m.; and 4 p.m. to 12 midnight. The concentration of riboflavin in the whole eight-hour specimen is compared with that in the sample taken within each eight-hour urine collection period.

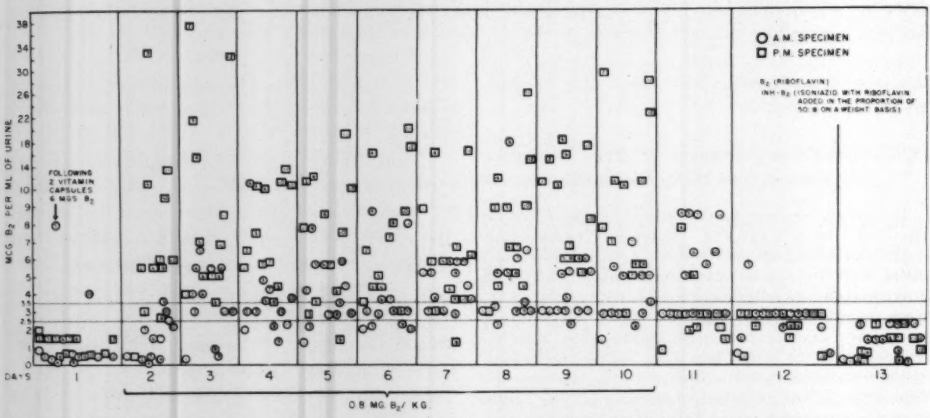
RIBOFLAVIN EXCRETION IN HOSPITALIZED PATIENTS RECEIVING INH-RIBOFLAVIN IN A SINGLE A.M. DOSE
A.M. SPECIMEN
P.M. SPECIMEN
MCG. B_2 PER ML. OF URINE
FOLLOWING 2 VITAMIN CAPSULES 6 MGS. B_2
DAYS
0.8 MG. B_2/ KG.

FIG. 5. The riboflavin concentration of urine specimens collected from 12 hospitalized patients at 8 a.m. and 8 p.m. Riboflavin, 0.8 mg. per kg., was started on the second observation day and was stopped on the tenth day.

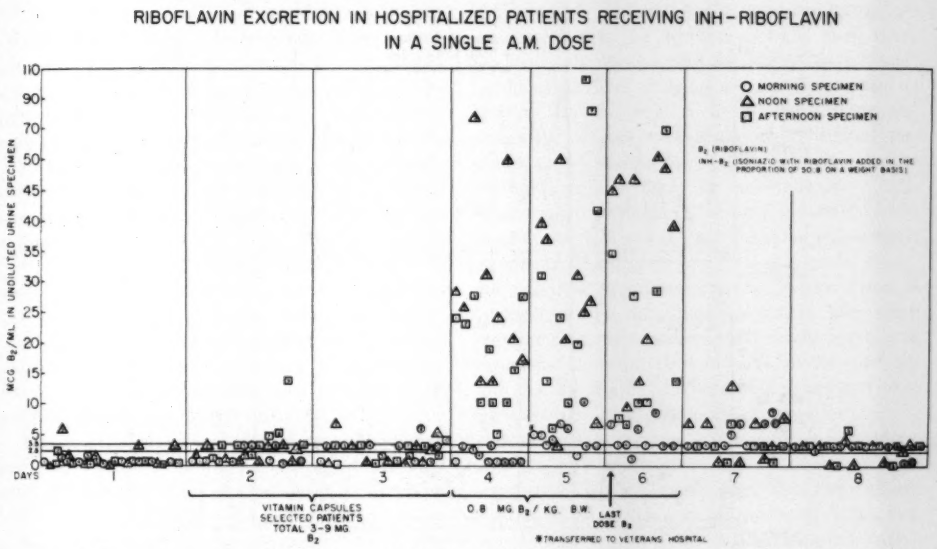
RIBOFLAVIN EXCRETION IN HOSPITALIZED PATIENTS RECEIVING INH-RIBOFLAVIN IN A SINGLE A.M. DOSE
MORNING SPECIMEN
NOON SPECIMEN
AFTERNOON SPECIMEN
MCG. B2/ML. IN UNDILUTED URINE SPECIMEN
DAYS
VITAMIN CAPSULES SELECTED PATIENTS TOTAL 3-9 MG. B2
0.8 MG. B2 / KG. B.W.
LAST DOSE B2
*TRANSFERRED TO VETERANS HOSPITAL

FIG. 6. Comparison of concentrations of riboflavin in urine specimens collected from 10 New York Hospital patients receiving standard hospital diet.

medication. Although several of these patients missed taking the drug occasionally, it was apparent that they were the ones who could be entrusted to administer their own medication. On the other hand, the patients represented by the riboflavin patterns in examples *No. 8* and *No. 9*, particularly the latter, clearly showed a high degree of irregularity in self-medication. While it is true that they might take the prescribed drug from time to time, they were sufficiently erratic in their drug administration to give the physician serious concern about their ability to follow a home-treatment regimen.

In Navajo domiciliary patients given riboflavin-tagged isoniazid, it was apparent from the urine riboflavin concentrations that some of the patients had taken the drug and others had not. However, since only 15 Navajo patients have been followed briefly, no final evaluation concerning regularity of drug self-administration is warranted. Only 10 of the first 18 urine specimens in which the riboflavin concentrations were determined showed concentrations of more than 2.5 γ per ml., indicating that these patients had taken their prescribed drug during the preceding fifteen to twenty-four hours. Further periodic serial urine riboflavin determinations will be required to determine whether the patients are regular or irregular followers of the self-administration drug regimen.

DISCUSSION

Comment has been made previously concerning the problems which arise when a physician is unaware that a patient has failed to take drug therapy according to prescribed regimen. Attention has been called also to the difficulties that are encountered in the detection of most therapeutic drugs in body fluids when specially staffed and specially equipped laboratories are not available.

In the present report, data have been presented which suggest that riboflavin, an easily detected compound, may be used as a means of identifying such therapeutic agents. Both isoniazid and riboflavin are readily absorbed from the gastrointestinal tract following oral administration, and are excreted in the urine. When isoniazid was administered in the form of oral tablets containing riboflavin as well, detection of riboflavin in the urine was accepted as evidence that isoniazid had also been ingested.

The importance of establishing, on repeated occasions, the concentration of riboflavin in the urine of each patient prior to the start of drug therapy cannot be overestimated if riboflavin is

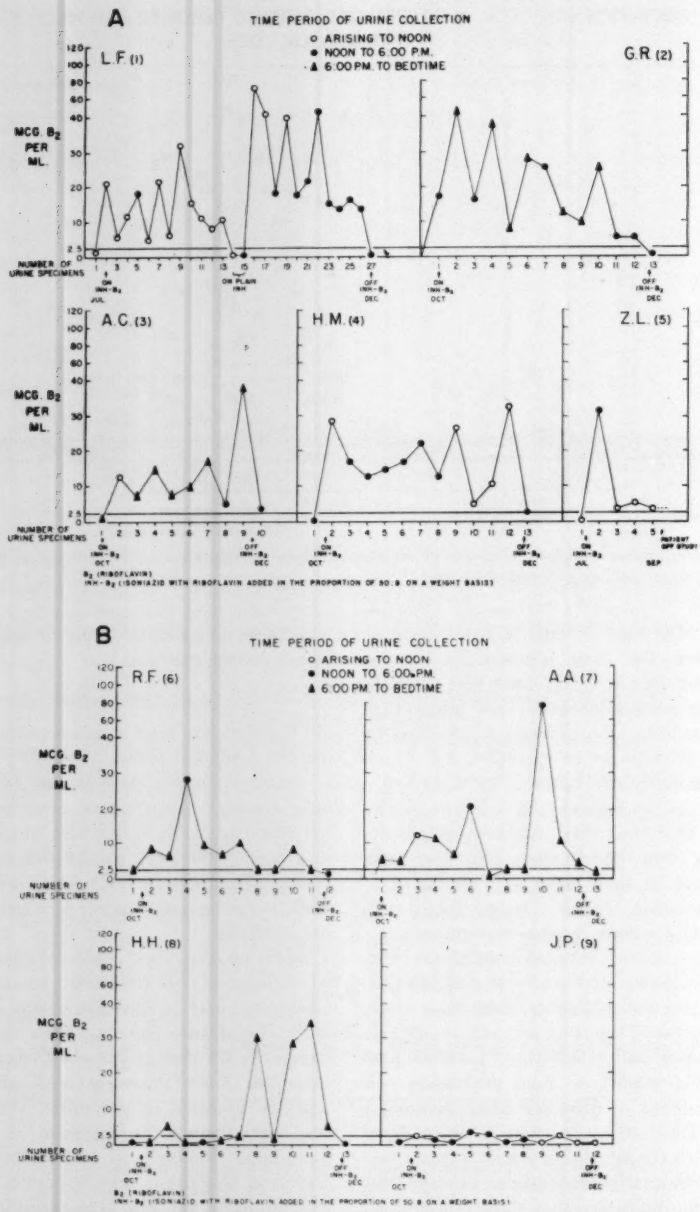

FIG. 7. The riboflavin concentration in the urine specimens from 9 New York domiciliary patients on riboflavin-tagged isoniazid medication (0.8 mg. riboflavin per kg. of body weight).

to be used as a measure of drug acceptance. Many persons habitually consume vitamin supplements and thus may normally excrete higher concentrations of riboflavin than others. The person who takes supplementary vitamins only sporadically, however, constitutes the real problem, for in him the concentration of riboflavin may vary widely. In the experiments described, the concentrations of riboflavin following administration of isoniazid with riboflavin added were well in excess of those usually observed after administration of vitamins in supplementary capsular form. It seems unlikely, therefore, that difficulty would be encountered in recognizing those who had accepted isoniazid-riboflavin therapy and those who merely had received supplementary vitamin therapy.

Vitamins in the form of dietary supplements are used most widely among persons in the high income countries. Yet, it is in societies in which low incomes prevail, as, for example, in the so-called underdeveloped countries of the world, that the need for determining regularity of drug self-medication is perhaps most important. In such situations, the use of vitamin supplements would rarely be a complicating factor.

Riboflavin, as mentioned previously (4), is a normal constituent of the body and rarely produces toxic effects. In the patients employed in the study described, and in the dosages used, no toxic manifestations were noted. There were no complaints, moreover, which would suggest that the tablets were unpalatable to the patients or that the color or odor of their urine was offensive to them.

The concentration of riboflavin in urine may be measured quickly and accurately by anyone accustomed to performing simple laboratory procedures, provided appropriate equipment is available. Although little equipment is needed, the cost and/or availability of a suitable photofluorometer at times may make use of the procedure unfeasible. In the writers' experience to date, instruments for detection of fluorescence in a qualitative manner only have not allowed differentiation between that degree of fluorescence produced by the normal riboflavin content of urine, that produced by riboflavin excreted due to supplementary vitamin intake, that produced by other fluorescing substances (as, for example, porphyrin) and that due to the administration of isoniazid with riboflavin added.

Ultimately simpler techniques requiring less equipment may be developed. Until such time, however, riboflavin may be useful as a "tag" for identification of drugs administered with it. It may be of special help in determining the regularity of self-medication in individual patients or in comparing groups of patients receiving isoniazid singly or in combination with other drugs. Ultimately, it may be desirable to employ riboflavin as a "tag" for other therapeutic agents. In this regard, however, it must be recognized that its incorporation into dosage forms of multiple drugs administered within the same hospital or to the same patient might negate its value, for it would then be difficult to establish which of the riboflavin-containing drugs had been ingested by any given patient.

Because there is now a potent drug armamentarium for a wide spectrum of diseases, it is imperative that we learn a great deal more about the extent to which self-administered drugs can be entrusted to the patient. If the characteristics of patients suitable for participation in a home-care treatment program can be defined, the delegation of patient responsibility for therapy could be put on a more rational basis.

While it is true that the practitioner can deliver in the home today a more effective antituberculous therapy than was available in the hospital ten years ago, the mere delivery of a therapeutic agent into the hands of the patient is no guarantee that the drug will be taken as prescribed.

The social scientist, in addition to the physician, may find the use of riboflavin helpful as a "tagging" substance for therapeutic drugs. Once it is established which patients do and which do not accept medication given without supervision, it may be possible for him to determine the extent to which language, cultural differences, the physician-patient relationship, or other factors influence the success or failure of a therapeutic program. Such information is greatly needed in ambulatory and domiciliary health programs within the United States and in international technical assistance programs as well.

Summary

Riboflavin has been incorporated into isoniazid tablets designed for antituberculous therapy in a proportion of 50:8 by weight. Measurement of the concentrations of riboflavin in the urine of

normal patients, of patients ingesting supplementary vitamin preparations, and in the urine of patients taking isoniazid with riboflavin added in this proportion has established that riboflavin may be used as a means of detecting those patients who have ingested isoniazid less than twenty-four hours prior to the test. In the experiments described herein, the concentration of riboflavin in normal urine was equal to or less than 2.5 γ per ml. in essentially all instances. The concentration in patients receiving isoniazid in dosages equivalent to 5 to 10 mg. per kg. of body weight, with riboflavin added, i. e., in dosages equivalent to 0.8 to 1.6 mg. per kg., exceeded 2.5 γ per ml.

The results of these studies indicate that riboflavin-tagged isoniazid is a practicable method for separating the patients who regularly follow self-medication programs from those who do not.

Sumario

Utilidad Clínica de la Isociandida Combinada con Riboflavina para Automedicación en los Tuberculosos

Se ha incorporado la riboflavina en tabletas de isoniacida destinadas a la terapéutica antituberculosa en una proporción de 50:8 por peso. La medición de las concentraciones de riboflavina en la orina de los enfermos normales, de los enfermos que ingieren preparaciones complementarias de vitaminas y en la orina de los enfermos que toman isoniacida con riboflavina en dicha proporción ha establecido que puede usarse la riboflavina como medio de descubrir a las personas que han ingerido isoniacida menos de veinticuatro horas antes de la prueba. En los experimentos aquí descritos, la concentración de riboflavina en la orina normal fué igual, o menor, de 2.5 γ por ml. esencialmente en todos los casos. La concentración en los enfermos que recibieron isoniacida a dosis equivalentes a 5 a 10 mg. por kg. de peso vivo, con la adición de riboflavina, o sea a dosis equivalentes a 0.8 a 1.6 mg. por kg. de peso, excedió de 2.5 γ por ml.

Sugieren estos estudios que la isoniacida combinada con riboflavina puede ser un método práctico para separar a los enfermos que emplean regularmente regímenes de automedicación de los que no hacen tal cosa.

Resume

Utilité en clinique de l'isoniazide-riboflavine "traçés" pour le self-traitement de malades tuberculeux

De la riboflavine a été incorporée dans des comprimés d'isoniazide préparés pour un traitement antituberculeux, dans la proportion de 50:8 par poids. La mesure de la concentration de la riboflavine dans l'urine de sujets normaux, de malades prenant des préparations supplémentaires de vitamines et dans l'urine de malades prenant de l'isoniazide, avec de la riboflavine additionnée dans cette proportion, a permis d'affirmer que la riboflavine peut être utilisée comme moyen de détecter les personnes qui ont pris de l'isoniazide moins de vingt quatre heures avant l'examen. Dans les expériences ici décrites, la concentration de riboflavine dans l'urine normale fut égale ou inférieure à 2,5 γ par ml dans essentiellement tous les cas. La concentration des malades recevant de l'isoniazide à des doses allant de 5 à 10 mg par kg de poids corporel, avec addition de riboflavine, c'est à dire à des doses allant de 0,8 à 1,6 mg par kg, fut supérieure à 2,5 γ par ml.

Ces études font penser que l'isoniazide "marquée" à la riboflavine peut être une méthode pratique pour séparer les malades qui suivent régulièrement leurs ordonnances de self-traitement de ceux qui ne le font pas.

Acknowledgment

The writers are indebted to the staff in The New York Hospital Chest Disease Pavilion and Chest Clinic; to the Many Farms staff of the Navajo-Cornell Field Health Research Project; and to Mrs. James Coatsworth for assistance in this study.

Thanks are also due to Miss Helen Ernest and Mrs. Dorothy Duerschner for technical assistance.

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KANAMYCIN[1, 2]

I. Treatment of Pulmonary Tuberculosis

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Introduction

Kanamycin, first isolated by Umezawa and his co-workers (1), is a basic, water-soluble compound of microbial origin. The known facts about this drug have recently been detailed by Finegold (2). The crystalline sulfate of kanamycin is poorly absorbed from the gastrointestinal tract, but readily absorbed when administered by intramuscular injection. The serum concentration falls rapidly in six to eight hours; a large proportion of the drug can be recovered in the urine within twenty-four hours. Kanamycin has been shown to inhibit the growth of gram-negative and gram-positive organisms, including mycobacteria, both *in vitro* and in experimental animals. Microorganisms resistant to kanamycin have been shown to be resistant to other antimicrobials. Tubercle bacilli resistant to viomycin are resistant to kanamycin, but the reverse is not the case; microorganisms resistant to kanamycin are resistant to neomycin, and vice versa. This cross resistance and the close resemblance in chemical structure suggested that there might be other similarities and that kanamycin and neomycin are indeed derivatives of the same series. Study of the effect of kanamycin in the experimental animal revealed a diminution in response to auditory stimuli and degenerative changes in the renal tubules similar to the known toxic effects of neomycin.

Donomae (3, 4) described the use of kanamycin in cases of acute, previously untreated pulmonary tuberculosis. He considered 4 gm. per week to be necessary, but 6 gm. per week to be the optimal therapeutic dose. In this study, when the drug was given with para-aminosalicylic acid (PAS), the therapeutic effect, as judged by the rate of sputum "conversion" and roentgenographic improvement, was considered comparable to that of streptomycin and PAS. Little loss of susceptibility to the drug was demonstrated in tubercle bacilli isolated during five months of therapy. Ototoxicity did not constitute a problem at a dosage of less than 6 gm. per week. The therapeutic and toxic effects have been observed in cases of chronic, previously treated pulmonary tuberculosis (5–8). In these cases there was little improvement, and manifestations of toxicity were universally encountered. Eosinophilia of the peripheral blood and transient elevation of the blood urea nitrogen with proteinuria and granular casts in the urine were common. The nephrotoxic effect has been an important complication, particularly in patients with pre-existing renal disease (9–11). Ototoxicity, particularly in the presence of impaired renal function, limits the safe administration of the drug to relatively short periods of time (11–21). The early loss of microbial susceptibility has been the common experience (4–8).

This report includes an evaluation of the therapeutic effect of kanamycin in the treatment of pulmonary tuberculosis, the appearance of microbial resistance during treatment, and the limitations imposed by toxic effects of the drug.

Case Material and Methods

Treatment with kanamycin was instituted in 36 patients, the aim being to continue therapy for a period of four months.

The patients were divided into three groups. In *Group I*, which contained 17 patients, observation of toxic effects of the drug and study of the emergence of microbial resistance to it were the chief objectives. The group consisted of 10 men and 7 women; ages ranged from thirty to sixty-eight years, with an average of forty-two years. Ten were Negro, 7 were white. All had chronic, moderately to far advanced disease. All had received extended courses of chemotherapy in the past and were assumed to harbor tubercle bacilli resistant to the three standard drugs. The dosage of kanamycin ranged from 0.5 gm. to 1.5 gm. daily in half-gram doses, or 10 to 30 mg. per kg. of body weight. The duration of treatment was ten to eighteen weeks.

[1] From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York; and the Chest Service, Bellevue Hospital, New York.

[2] This study was supported by a grant from Bristol Laboratories, Inc., Syracuse, New York, who also supplied the kanamycin (Kantrex).

In the other two groups, evaluation of the therapeutic efficacy was the chief objective; study of toxic effects and of the emergence of bacterial resistance was continued. Seven patients were included in *Group II*: 6 men and one woman, with ages ranging from twenty-three to fifty-two (an average of forty-two years). Six were Negro and one was white. All had active, previously untreated disease with an acute component in all cases, far advanced in 6 and moderately advanced in one. They received 1 gm. of kanamycin daily in divided doses, or 15 to 23 mg. per kg. of body weight. Therapy was continued for six to seventeen weeks.

Group III contained 12 patients, 9 men and 3 women, ages ranging from twenty-three to sixty-eight (an average of forty years). Four were Negro, 8 were white. As in *group II*, all of the patients had active, acute, previously untreated disease. They were given 1 gm. of kanamycin three times weekly, or 12 to 26 mg. per kg. of body weight on each of these days. Alternate cases, namely 6 patients, also received PAS, 15 gm. daily. These subgroups will be referred to as "kanamycin" and "kanamycin–PAS" patients, respectively. In each subgroup, the disease was far advanced in 3 patients and moderately advanced in 3. The duration of treatment was ten to sixteen weeks.

These patients were closely followed by clinical, bacteriologic, and roentgenographic observation; laboratory tests were repeated at regular intervals. The body weight and the result of urinalysis were recorded at weekly intervals. Sputum volume was measured daily. The peripheral blood was examined every two weeks for hemoglobin content, total leukocyte and differential counts. In *Group I*, the blood urea nitrogen was determined at intervals of two weeks; in *Groups II* and *III*, once weekly. Tests of liver function were repeated at monthly intervals. Prior to treatment, lateral and oblique roentgenograms were obtained in each case in addition to the standard posteroanterior film; the last was repeated once every month. Microscopic examination of sputum and culture of sputum or gastric contents were repeated at monthly intervals. Audiometric examinations were made by members of the Department of Otolaryngology of Bellevue Hospital once a month in the patients in *Group I*, and weekly in the patients in *Groups II* and *III*.

Results

Therapeutic Effect

In *Group I*, because of the nature of the disease, there was little opportunity to evaluate therapeutic efficacy. The preliminary results of this study have been reported (7). Eleven of the 17 patients completed four months of therapy, and all but one completed three months. Little clinical improvement was observed. Only one patient had a decrease in the volume of sputum. There was no roentgenographic improvement in 10 patients, in 6 there was slight improvement, and in one there was progression of the disease. The sputum of 15 patients continued to contain tubercle bacilli on microscopy and culture. No tubercle bacilli were isolated from one patient before or during treatment, and, in one, none were demonstrable by microscopy or culture after four months of therapy. One patient had a resection during the course of treatment; the right upper lobe, containing a peripheral carcinoma, was removed. This lobe also contained most of the tuberculous disease in this patient; the postoperative course was uncomplicated.

The more important aspects of the therapeutic effect observed in *Groups II* and *III* have been included in tables 1–3. Of the 7 patients in *Group II*, treatment was continued for four months in 2, three months in 2, and two months in 2. In one case, therapy was discontinued early in the second month because of a severe degree of hearing loss. Treatment was stopped prematurely in all 5 patients because of evidence of developing ototoxicity. One of these patients also had clinical and roentgenographic evidence of progression of the disease. All of the 7 patients had acute disease and were febrile prior to treatment. In 6, there was reduction of temperature to normal levels within six weeks. There was also an improvement in the sense of well-being of 6 patients. Five patients gained at least 5 pounds, and 5 had decreased cough and reduction in the volume of sputum. Serial roentgenograms demonstrated improvement of the disease which was regarded as substantial in one patient, moderate in 3, and slight in one. In one patient there was no change, and in one there was progression of the disease. Cavities were reduced in size in 4 of the 5 patients in whom these were present prior to treatment. In no case was there evidence of cavity closure. The sputum of 5 patients became negative for acid-fast bacilli on microscopy between five and twelve weeks after the start of treatment, and in one of these patients there was no growth of tubercle bacilli on culture after five weeks. The sputum of 2 patients persistently contained tubercle bacilli on microscopy and culture.

Of the 12 patients on intermittent therapy in *Group III*, 9 completed the prescribed course. The treatment of 2 kanamycin patients was discontinued after three months; of these, one left

TABLE 1

GROUP II—MICROBIAL SUSCEPTIBILITY

Patient	Total Treatment	Clinical Course	Roentgengenographic Change	Sputum End of Treatment		Test of Microbial Susceptibility		
				Micros-copy	Culture	Before Treatment	During Treatment	
							First	Second
	(gm.)							
A. C.	52	Improved	Moderate improvement	−	+	Not tested	Not tested	Not tested
I. C.	66	Worse	No change	+	+	Susceptible	Complete resistance (66 gm.)	
M. G.	120	Improved	Moderate improvement	+	+	Slight resistance	Intermediate resistance (62 gm.)	Complete resistance (91 gm.)
C. J.	95	Improved	Substantial improvement	−	+	Slight resistance	Intermediate resistance (64 gm.)	Complete resistance (91 gm.)
J. L.	81	Worse	Worse	−	+	Slight resistance	Complete resistance (42 gm.)	
E. S.	97	Improved	Moderate improvement	−	+	Not tested	Slight resistance (42 gm.)	Slight resistance (71 gm.)
C. T.	40	Improved	Slight improvement	−	+	Not tested	Slight resistance (39 gm.)	Complete resistance 2 months after treatment stopped

− = Negative reaction.
+ = Positive reaction.
Susceptible = Less than 2+ growth in 10 γ per ml.
Slight resistance = 2+ growth or more in 10 γ per ml.
Intermediate resistance = 2+ growth or more in 50 γ per ml.
Complete resistance = 2+ growth or more in 500 γ per ml.

TABLE 2

GROUP III—KANAMYCIN: MICROBIAL SUSCEPTIBILITY

Patient	Total Treatment	Clinical Course	Roentgenographic Change	Sputum End of Treatment		Test of Organism Susceptibility		
				Microscopy	Culture	Before Treatment	During Treatment	
							First	Second
	(gm.)							
J. D.	40	Worse	Worse	+	+	Slight resistance	Slight resistance (18 gm.)	Slight resistance (40 gm.)
F. P.	53	Worse	Worse	+	+	Slight resistance	Slight resistance (21 gm.)	Complete resistance (53 gm.)
M. R.	52	Slight improvement	Worse	+	+	Susceptible	Slight resistance (14 gm.)	Complete resistance (30 gm.)
J. S.	53	Slight improvement	Moderate improvement	−	+	Slight resistance	Not tested	Not tested
A. C.	37	Moderate improvement	Moderate improvement	−	+	Not tested	Slight resistance (12 gm.)	Not tested
S. B.	52	Moderate improvement	Worse	+	+	Not tested	Slight resistance (9 gm.)	Complete resistance (21 gm.)

See legends to table 1.

TABLE 3

GROUP III—KANAMYCIN-PAS: MICROBIAL SUSCEPTIBILITY

Patient	Total Treatment	Clinical Course	Roentgenographic Change	Sputum End of Treatment		Test of Organism Susceptibility		
				Microscopy	Culture	Before Treatment	During Treatment	
							First	Second
	(gm.)							
G. F.	52	Moderate improvement	Moderate improvement	−	+	Susceptible	Slight resistance (15 gm.)	Slight resistance (52 gm.)
R. M.	54	Moderate improvement	Slight improvement	−	+	Slight resistance	Slight resistance (54 gm.)	Not tested
W. S.	53	Moderate improvement	Moderate improvement	−	−	Slight resistance	Slight resistance (20 gm.)	Culture negative
A. S.	53	Moderate improvement	Moderate improvement	−	+	Susceptible	Slight resistance (22 gm.)	Slight resistance (53 gm.)
A. P.	52	Moderate improvement	Substantial improvement	−	−	Not tested	Slight resistance (18 gm.)	Culture negative
F. F.	38	Moderate improvement	Moderate improvement	−	+	Slight resistance	Not tested	Not tested

See legends to table 1.

the hospital against advice, and one had roentgenographic evidence of spread of the disease. The last patient had evidence of developing ototoxicity at the same time. One kanamycin–PAS patient left the hospital against advice after three months of treatment. Four of the 6 kanamycin patients were febrile before treatment, and in all of these the temperature returned to normal. There was improvement in the sense of well-being of one kanamycin patient and all 6 kanamycin–PAS patients. Three of the kanamycin patients and 5 of the kanamycin–PAS patients had gains in weight of at least 5 pounds. Cough decreased and the volume of sputum was reduced in 5 of the 6 patients in each of the subgroups. Two kanamycin patients had a moderate degree of improvement in the disease as evidenced by serial roentgenograms, and in both there was diminution in cavity size. Four kanamycin patients had evidence of progression of the disease, although in one there was some decrease in cavity size. In all 6 kanamycin–PAS patients there was roentgenographic evidence of improvement; in one patient this was considered to be substantial; in 4, moderate; and in one, slight. Cavities had previously been demonstrated in all 6 patients, and decrease in cavity size was observed in 4. Evidence of cavity closure was not observed. In 2 kanamycin patients, tubercle bacilli disappeared from the sputum (by microscopy only) after six and ten weeks of treatment, and in all 6 of the kanamycin–PAS patients after four to eight weeks. Tubercle bacilli continued to grow on culture of the sputum of all of the kanamycin patients; in 2 of the kanamycin–PAS patients tubercle bacilli could no longer be isolated on culture after six and sixteen weeks of treatment.

After the course of kanamycin, treatment using a combination containing isoniazid was continued in all of the patients in *Groups II* and *III*. In those patients in whom the response to treatment had been poor, as judged by roentgenographic change, the second course of chemotherapy produced substantial improvement. In patients in whom there had already been good response to treatment, further resolution of the disease was less pronounced.

Microbial Susceptibility

Drug susceptibility was determined by growth of tubercle bacilli in liquid medium containing 10, 50, and 500 γ of kanamycin per ml. Resistance was classified as slight, intermediate, or complete, corresponding to a two-plus growth or more in each of these three dilutions.

Studies are available from 11 patients in *Group I*. The strains of tubercle bacilli isolated from 4 patients before treatment were susceptible to the drug. In one, this state persisted after 50 gm., in 2 there was slight resistance after 5 and 14 gm., and this was unchanged after 70 and 126 gm., respectively. Tubercle bacilli isolated from one patient were completely resistant after 52 gm. of kanamycin. In 7 patients there was slight resistance before therapy. In 2 this was unchanged after 98 and 140 gm., and in 5 patients there was complete resistance at 136 gm.

Serial testing of microbial susceptibility was not carried out in all of the cases in *Groups II* and *III*. The available results are included in tables 1–3, allowing comparison with the clinical course, changes noted in serial roentgenograms, and the result of sputum examination at the end of treatment. In *Group II*, there was complete drug resistance after 42 to 91 gm. in 4 of the 6 patient-strains tested serially during treatment. In one patient-strain (*C. T.*) there was slight kanamycin resistance at the end of treatment (40 gm.), and complete resistance two months after discontinuation of therapy. In the kanamycin group there was complete kanamycin resistance after 21 to 53 gm. in the strains from 3 of the 4 patients on whom complete information was available; in the kanamycin–PAS group such complete resistance was not observed. Loss of susceptibility to PAS was demonstrated in one patient in whom the strain isolated at the end of treatment had intermediate resistance to kanamycin. The limited information available can only suggest a correlation between drug susceptibility and the efficacy of treatment. In *Group II*, in the patients treated with 1 gm. daily, there was rapid development of resistance. Tubercle bacilli from patients treated with 1 gm. three times weekly appeared to develop resistance after a lower dosage than did the bacilli of those patients given 1 gm. daily. When the intermittent dosage was combined with PAS, there was little evidence of developing resistance.

Illustrative Cases

Three cases are described in detail in order to demonstrate and compare the best results ob-

tained with each of the three regimens employed in the treatment of patients with acute disease.

Case 1. (*C. J.*), a 23-year-old Negro male, had a history of recent respiratory and constitutional symptoms. There was roentgenographic evidence of extensive bilateral disease involving mainly the left upper and the right lower lobes, with large cavities in both. He was treated with 1 gm. of kanamycin daily, with subsequent reduction in the severity of cough and volume of sputum. His general condition improved, with disappearance of fever and a gain in weight of 12 pounds. Tubercle bacilli disappeared from the sputum on microscopy after one week of treatment, but continued to grow on culture. Organisms isolated before treatment were slightly resistant to kanamycin; there was intermediate resistance after 64 gm. and complete resistance after 91 gm. Treatment was discontinued after 95 gm. because of audiometric evidence of developing loss of hearing. The roentgenographic change after treatment is demonstrated in figures 1 and 2. There was resolution of the disease in both lungs, with considerable diminution in the size of the cavity in the apex of the right lower lobe. This improvement was regarded as substantial.

Case 2. (*J. S.*), a 28-year-old Puerto Rican male, presented with mild symptoms of short duration. There was roentgenographic evidence of recently acquired disease in the apical and posterior segments of the right upper lobe, with a small cavity in the posterior segment. He was treated with kanamycin, 1 gm. three times a week. The severity of the cough and the volume of sputum were reduced. There was symptomatic improvement with a weight gain of 7 pounds. Tubercle bacilli disappeared from the sputum smear after six weeks of treatment, but continued to grow on culture. Tubercle bacilli isolated prior to therapy were

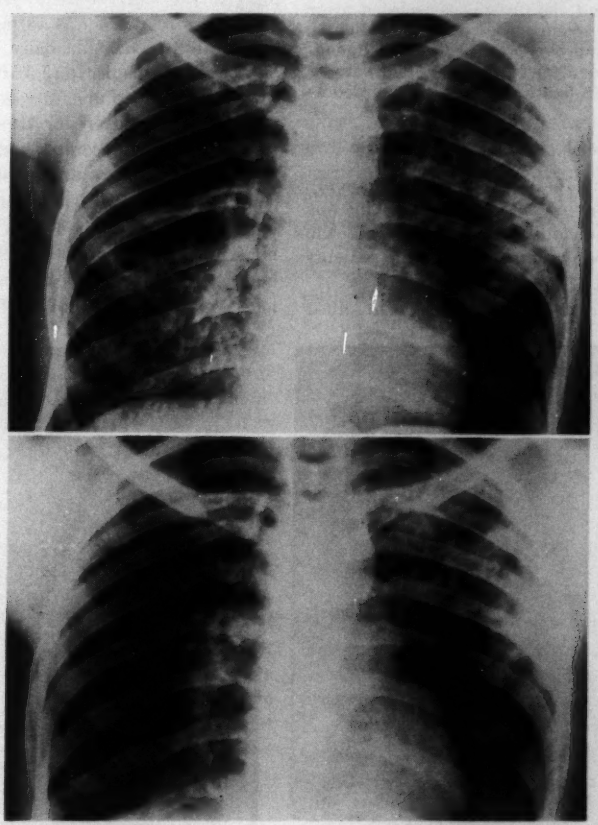

FIG. 1 (Top). Case 1 (*C. J.*) before treatment.
FIG. 2 (Bottom). Case 1 (*C. J.*) after treatment totaling 95 gm. of kanamycin.

slightly resistant to kanamycin; further tests of susceptibility were not obtained. Treatment was continued for the prescribed four months, during which the patient received 53 gm. of kanamycin. The roentgenographic change after treatment is demonstrated in figure 3. There was resolution of the disease with diminution in the size of the cavity. This improvement was regarded as moderate.

Case 3. (*A. P.*), a 23-year-old Puerto Rican female, presented with a history of respiratory symptoms, fever, and weight loss of recent onset. The disease was limited to the upper division of the left upper lobe. She was treated with kanamycin, 1 gm. thrice weekly, together with daily PAS. There was prompt improvement, with reduction in the volume of sputum, early loss of the low-grade fever, and a weight gain of 13 pounds. Tubercle bacilli could no longer be found in the sputum smear after five weeks, and ceased to grow on culture after six weeks. Only one susceptibility test was done, and the organisms isolated after administration of 18 gm. of the drug were slightly resistant. The improvement in the roentgenographic appearance after treatment, which totaled 52 gm., is shown in figure 4. This was regarded as substantial.

Complications

Pain at the site of injection was commonly experienced by the patients in *Group I*. This was alleviated by the addition of procaine to the kanamycin solution administered to the patients in *Groups II* and *III*.

Of the 24 patients on daily treatment, most had transient eosinophilia in the peripheral blood. Transient proteinuria was found in half of the patients in whom this had not been present before treatment, and granular casts were found in the urine in every case. Of the 12 patients on intermittent therapy, there was no eosinophilia, a trace of proteinuria was detected in 3, and urinary casts were found in 8. In several patients in each group, the blood urea nitrogen was observed to rise temporarily to between 20 and 25 mg. per 100 ml.; in all of these patients it returned to completely normal levels even though therapy was continued. Only one patient developed a significant degree of nitrogen retention. This patient (*C. T.*) was a 52-year-old Negro male who was a diabetic and had a variable degree of proteinuria and a few hyaline casts in the urine prior to the start of therapy. At this time, the blood urea nitrogen was 18 mg. per 100 ml. He was treated with 1 gm. daily, or 15 mg. per kg. of body weight per day. After two weeks of therapy, the blood urea nitrogen was 28. Routine studies were omitted for three weeks; by that time the patient complained of tinnitus, the presence of a severe degree of hearing loss was confirmed by audiometric examination, and the blood urea nitrogen had risen to 82 mg. per 100 ml. The drug was discontinued after the patient had received 40 gm. The clinical condition, which had deteriorated, improved following cessation

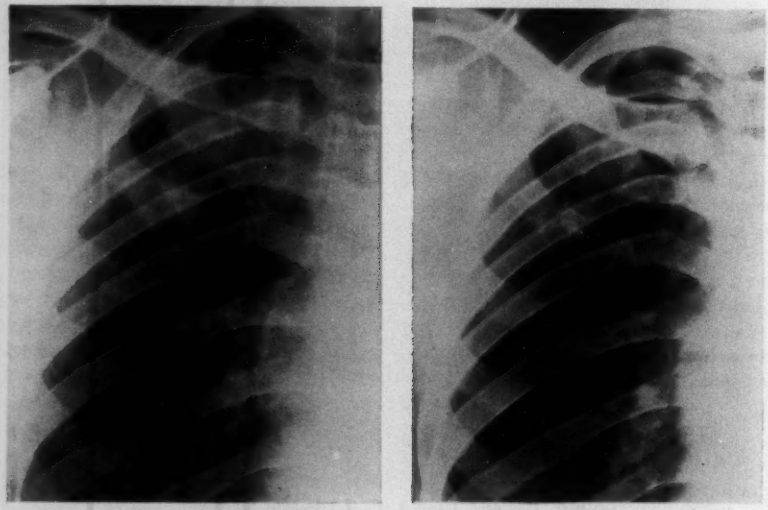

FIG. 3. Case 2 (*J. S.*) before and after treatment totaling 53 gm. of kanamycin.

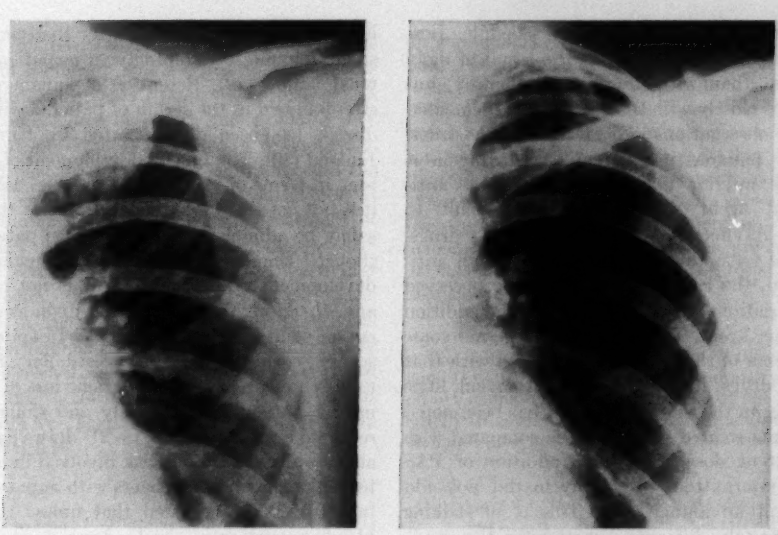

FIG. 4. Case 3 (*A. P.*) before and after treatment totaling 52 gm. of kanamycin–PAS.

of therapy. However, at the same time, the impairment of hearing progressed to complete deafness. He developed ataxia, though tests of vestibular function were normal. There were no abnormal neurologic signs. The blood urea nitrogen gradually approached normal, but remained at 47 mg. per 100 ml. five months after discontinuation of therapy.

The ototoxic effects, as observed in the present patients, are presented in detail by Frost and his associates (14). Twenty-one of the 24 patients receiving daily kanamycin were studied; impairment of hearing was detected in 17 of them after 32 to 134 gm. The other 4 patients had no impairment at the end of therapy, which totaled 59 to 113 gm. All of the 12 patients on intermittent therapy were closely observed; there was evidence of impairment of hearing in 2 after 37 to 54 gm. The other 10 patients had normal hearing when treatment was discontinued after 38 to 53 gm. The patients who developed a significant degree of hearing loss or a significant increase in pre-existing impairment frequently complained of tinnitus. There was no correlation between impairment of hearing and the transient increase in blood urea nitrogen observed in some patients.

DISCUSSION

The observations on the patients in *Group II*, to whom daily kanamycin was administered alone, establish that this is an effective agent in the treatment of pulmonary tuberculosis. In this small group of previously untreated patients with acute disease, the degree of improvement observed is more than could be expected from a program of modified bed rest without chemotherapy. The best results obtained were in those patients treated with PAS in addition to 1 gm. of kanamycin thrice weekly. However, as in all studies in which combined chemotherapy is employed, the effect of a single drug is difficult to evaluate, and both the therapeutic effect of PAS and its action in delaying the appearance of kanamycin resistance must be considered. The intermittent dosage, when used alone, appeared to be much inferior to either of the other regimens.

The duration of effective chemotherapy was limited by loss of microbial susceptibility and by the development of signs of ototoxicity. Microbial resistance, in terms of total dosage, was least in the patients on combined intermittent therapy; its earlier development in patients treated with intermittent kanamycin alone is in keeping with the poor therapeutic effect. In *Groups II* and *III* there was some correlation between the early emergence of resistant organisms and failure to improve, or progression of the disease. There was little difference in the total amount of drug which produced impairment

of hearing, whatever the dosage schedule. Loss of hearing occurred earlier as the result of daily treatment than with intermittent therapy, but correlated closely with total dosage.

These observations indicate that in the treatment of pulmonary tuberculosis kanamycin is certainly inferior to isoniazid, the best antimicrobial agent available. It is difficult to compare its effectiveness with that of other drugs. It would seem that the more effective an antimicrobial, the less difference would be observed from variations in dosage and from the addition of another agent. This concept may permit some comparison of the effect of kanamycin with that of other drugs. In the case of streptomycin, little difference in the short-term effect has been noted in patients treated with daily, as compared with intermittent dosage (22); the addition of PAS to the intermittent streptomycin did not add significant advantage (23). This is in striking contrast to the differences observed in this study between the use of kanamycin daily and its intermittent use, with and without PAS. The difference resulting from the use of daily and intermittent viomycin (24) was also less than that observed with kanamycin, but more than in the case of streptomycin. These facts suggest that kanamycin is inferior to both streptomycin and viomycin. The available reports of clinical experience with neomycin (25–28) contain few cases of acute untreated disease comparable to those in *Groups II* and *III* of this report. In these patients the daily dosage and duration of treatment were variable, so that precise comparison with the results observed in this study is not possible. There was, however, an occasional patient with acute disease in whom treatment with neomycin resulted in improvement comparable to that observed with kanamycin.

The common occurrence of ototoxicity seriously limits the usefulness of kanamycin in the treatment of tuberculosis. There has seldom been any recovery from this loss after discontinuation of the drug, although a mild degree of improvement has been observed (14, 15). Where information is available, progressive deterioration after cessation of therapy has been encountered only in patients in whom there has been impairment of renal function prior to therapy, with further deterioration after the administration of kanamycin (18–20). In these, gross impairment of hearing was already present at the time therapy was discontinued. Evidence of progressive ototoxicity has also been encountered after treatment with small amounts of streptomycin in patients with renal failure (29). Retention of kanamycin and streptomycin in the serum of patients with renal disease has been demonstrated by Kunin and Finland (30). The time of development of hearing loss, in terms of total dosage, is so variable that it is not possible to predict the amount that may safely be administered to any given patient. Although tinnitus commonly accompanies the development of hearing loss, it is not constant and, therefore, cannot be relied upon as an indication of developing ototoxicity. In spite of the severe cochlear damage, vestibular function usually remains intact. The tragic loss of hearing in one patient in this study and similar cases reported by others (11, 13, 18–21) draws particular attention to the serious risk involved in administering kanamycin to patients with impaired renal function. It is suggested that unless the daily blood concentration can be determined and can be controlled, the use of kanamycin under these circumstances for the treatment of tuberculosis constitutes an unjustifiable hazard. There is continuing need for effective chemotherapy during the immediate postoperative period in patients whose disease is chronic, and in whom organisms are resistant to the standard drugs. In patients whose renal function is normal and remains so, and in whom weekly audiometric examinations are available, kanamycin may safely be employed in a dose of 1 gm. daily. Evidence of ototoxicity will usually be delayed beyond the time at which postoperative complications are most common. It is thought that, in the treatment of pulmonary tuberculosis, kanamycin will be of most value in this group of patients. Viomycin is commonly employed in their postoperative management; comparison of its effect with that of kanamycin in preventing complications would be of great interest. Because of the loss of susceptibility to kanamycin in organisms resistant to viomycin, kanamycin will be ineffective in the treatment of patients who have previously received prolonged treatment with viomycin. There may be a place for kanamycin in the immediate postoperative period, reserving viomycin for more extended therapy.

Summary

Seventeen patients with chronic pulmonary tuberculosis were treated with kanamycin in order to observe toxic effects and the develop-

ment of microbial resistance to the drug. The therapeutic efficacy was studied in 19 patients with acute, previously untreated disease. A definite effect was observed, but probably less than that of either streptomycin or viomycin. The early loss of microbial susceptibility and the common occurrence of ototoxicity strictly limit the usefulness of the drug in the treatment of pulmonary tuberculosis. The possibility of preexisting renal disease constitutes a particular risk in connection with its use, because of the increase in the degree of impairment and the rapid development of ototoxicity under these circumstances. It is thought that the drug will be of most use in the early postoperative treatment of patients who harbor organisms resistant to the standard drugs.

Sumario

La Kanamicina: I. Tratamiento de la Tuberculosis Pulmonar

A 17 enfermos de tuberculosis pulmonar crónica se les trató con kanamicina a fin de observar los efectos tóxicos y la formación de resistencia microbiana a la droga. La eficacia terapéutica fué estudiada en 19 sujetos que tenían enfermedad aguda sin tratamiento anterior. Se observó efecto bien definido, pero probablemente menor que el de la estreptomicina o la viomicina. La pérdida temprana de la susceptibilidad microbiana y la aparición común de ototoxicidad limitan rígidamente la utilidad de la droga en el tratamiento de la tuberculosis pulmonar. La posible preexistencia de nefropatía constituye un riesgo especial en relación con su empleo, debido a acrecentar la intensidad de la incapacidad y a la rápida aparición de ototoxicidad en dichas circunstancias. Parece que el medicamento encontrará su mayor aplicación en el tratamiento postoperatorio temprano de los enfermos que albergan microbios resistentes a las drogas aceptadas.

Resume

Kanamycine. I. Traitement dans la tuberculose

Dix-sept malades présentant une tuberculose pulmonaire chronique furent traités avec de la kanamycine dans le but d'observer les effets toxiques et le développement d'une résistance du germe vis à vis du médicament. L'efficacité thérapeutique a été étudiée chez 19 malades ayant une forme aigüe, non traitée auparavant. Les auteurs observèrent un effet net mais probablement moindre que celui de la streptomycine ou de la viomycine. La perte précoce de la sensibilité du germe et la fréquence commune de la toxicité pour l'ouïe, limitent strictement l'utilité de ce médicament dans le traitement de la tuberculose pulmonaire. La possibilité d'une maladie rénale préexistente constitue un danger particulier dans son emploi, à cause de l'augmentation du degré d'altération et du développement rapide des effets toxiques sur l'ouïe, dans ces circonstances. On pense aussi que ce médicament sera surtout utilisé dans le traitement précoce préopératoire de malades porteurs de germes résistant aux médicaments courants.

Acknowledgments

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KANAMYCIN[1, 2]

II. Ototoxicity

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Introduction

Kanamycin has been in general clinical use for more than one year, and has proved of value in infections due to a wide variety of organisms, particularly staphylococci, *E. coli*, and the aerobacter and klebsiella groups (1). It has also been used as an antituberculous agent (2–4). The major difficulty encountered in the administration of the drug has been its toxic effect on the eighth nerve, particularly on the cochlear division. Since kanamycin closely resembles neomycin in both chemical configuration and antimicrobial activity, this property is not a surprising one. Both substances contain 2-deoxystreptamine attached by glycosidic linkage to aminohexoses, and both show similar activity against *M. tuberculosis* to the point of demonstrating complete cross resistance.

Preliminary animal experiments (5) suggested that kanamycin was less toxic to the auditory system than dihydrostreptomycin. Subsequent clinical investigations (3, 6–9) revealed that the incidence and severity of damage to the auditory system of man was greater than the animal experiments had suggested. In addition, several cases have been reported in which hearing loss was rapid in onset, progressive, and severe (7–15), occurring in some instances after administration of small amounts of the drug (10–14). Usually these were patients in whom renal damage was present. Several investigators have drawn attention to the fact that patients with impaired renal function are particularly vulnerable to the ototoxic effects of kanamycin (6–8, 10–12, 16, 17).

The present study was undertaken in cooperation with the Bellevue Hospital Chest Service under Dr. J. H. McClement. The patients in the study were undergoing long-term treatment for pulmonary tuberculosis, and this provided the opportunity to investigate the effects of the drug over a prolonged period. While progress reports have been published during the past year (7, 8, 18), this paper presents a final report of the ototoxic effects of kanamycin in these patients.

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[2] This study was supported by a grant from the Alfred P. Sloan Foundation.

Material and Methods

The case material consisted of 33 of the 36 patients in the Chest Service kanamycin study. These patients were divided into three groups according to the treatment instituted by the Chest Service and described in their report (19). Three patients in the first group were not included in the present study because 2 were too ill to give reliable audiometric measurements, and one was excluded because he had received viomycin as well as kanamycin.

Prior to treatment, an otologic examination, an audiogram, and a caloric test for vestibular function were done on each patient. During treatment, an audiogram was done at monthly intervals on patients in *Group I* and at weekly intervals on patients in *Groups II* and *III*. Following treatment, a hearing test was done each week for four weeks and monthly thereafter as long as the patient was available. Vestibular function was also tested at the termination of treatment.

In *Group I*, kanamycin was continued beyond the trial period of one hundred and twenty days so long as the patient's tuberculous condition appeared to be improving. It was not stopped in several patients until loss of hearing had progressed to involve several frequencies. In *Groups II* and *III* it was stopped as soon as hearing loss was detected or, otherwise, after one hundred and twenty days of treatment.

Results

Hearing Loss

Approximately half of the patients had normal audiograms at the beginning of treatment: 7 of the 14 patients in *Group I*, 4 of the 7 patients in *Group II*, and 6 of the 12 patients in *Group III*. The remainder showed a perceptive loss of hearing, usually of mild degree. In 2 patients the loss was mixed, both conductive and perceptive.

A loss of 15 decibels or more from the pre-

TABLE 1

INCIDENCE OF OTOTOXICITY

Group	Gm. per Day	Total Dose	Number of Patients Treated	Hearing Loss	Tinnitus	Vestibular Symptoms	No Change
		(gm.)					
I	0.5	59–61	3	1	1	—	2
	1.0	112–163	8	8	4	—	—
	1.5	143–149	3	3	3	—	—
II	1.0	39–120	7	5	4	1	2
III	3.0 per week	37–54	12	2	1	—	10

treatment level was regarded as significant. The incidence of ototoxicity in each group of patients is shown in table 1. In *Group I* (those patients receiving 0.5 to 1.5 gm. daily), 12 of the 14 patients showed a significant loss; in *Group II* (those receiving 1 gm. daily), 5 of the 7 patients showed a significant loss; and in *Group III* (those receiving 3 gm. per week), 2 of the 12 patients showed a significant loss.

The distribution of patients according to the total dose at the onset of auditory disturbance is presented in table 2. The earliest hearing loss occurred after 32 gm. had been given to a patient who was receiving 0.5 gm. of kanamycin daily (patient *P. A.*). It is interesting to note that this patient had a mild perceptive loss before the start of treatment, which had developed following previous dihydrostreptomycin therapy. In those patients with normal hearing before treatment, the earliest loss noted was after 52 gm. (patient *A. C.*).

The manner in which the hearing loss progressed followed a definite pattern. The first frequency involved in all cases was 8,000 cycles per second. If the drug was continued, the other frequencies were involved in descending order. In all instances the loss was perceptive in type, and was bilateral in all but 3 patients. In these 3 the kanamycin was stopped immediately on the appearance of a slight loss at 8,000 cycles per second in the one ear. Usually the losses were equal in both ears, but occasionally one ear became impaired before the other.

As would be expected, the more frequencies that were involved the greater was the degree of deafness experienced by the patient. Losses confined to 8,000 cycles per second and 4,000 cycles per second were usually not noticed by the patient. As the "speech frequencies" (2,000, 1,000, and 500 cycles per second) became involved, the patient's ability to follow conversation progressively decreased.

Since the pattern of hearing loss was similar in all of the patients, it is possible to summarize the results in a series of formalized audiograms, as in figures 1 to 5. In each figure line A represents normal hearing, and lines B to G the increasing degrees of hearing impairment as successively lower frequencies become involved. Thus, B indicates a loss at 8,000 cycles per second only, C at 4,000 and 8,000 cycles per second, and so on to G, in which all frequencies are affected. Change in a patient's hearing level is shown by arrows (each representing a single ear) which begin (solid circles) at the line corresponding to the initial hearing level (neglecting conduction losses) and end at the line representing the final hearing level. Patients are indicated by initials, and the total number of grams of kanamycin given to each patient is noted below the arrows.

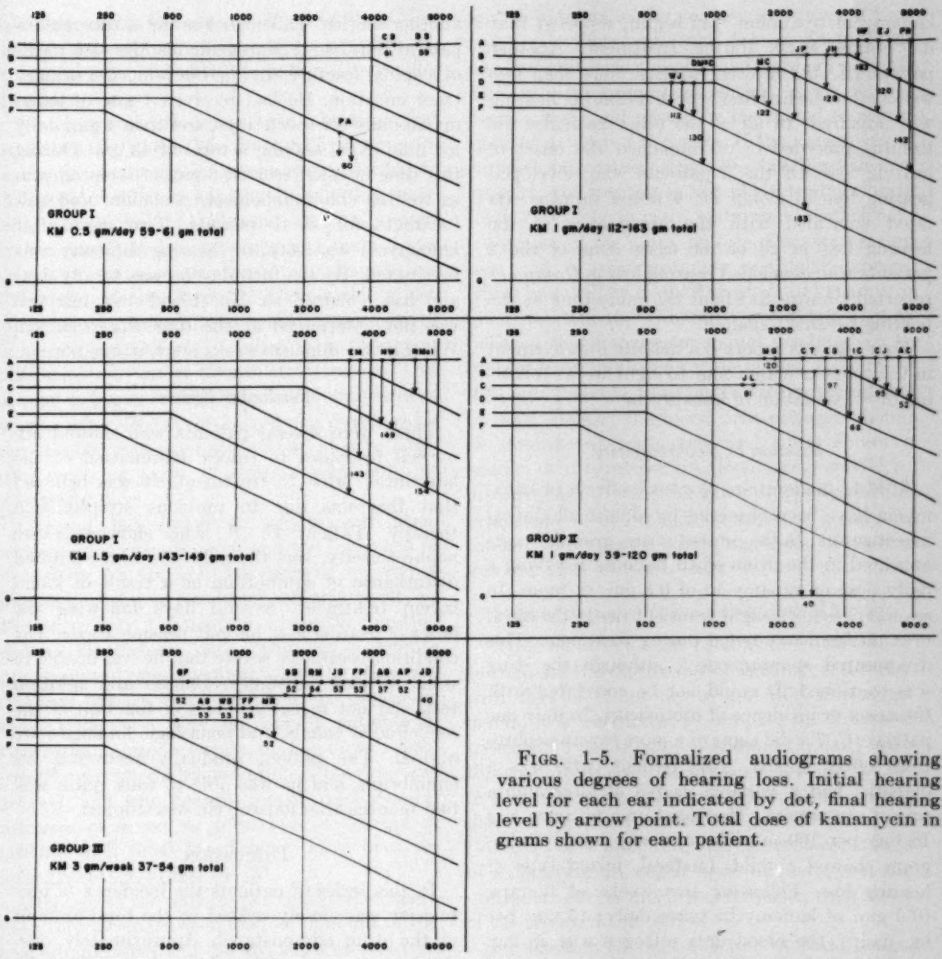
TABLE 2

DISTRIBUTION OF PATIENTS ACCORDING TO THE TOTAL DOSE AT THE ONSET OF AUDITORY DISTURBANCE

Group	Dose		Total Dose at Onset of Auditory Disturbance				
			(Gm.)				
	(Gm./Day)	(Mg./Kg.)	0–30	31–60	61–90	91–120	121–150
I	0.5	(10–13)	—	1	—	—	—
	1.0	(13–22)	—	2	1	4	1
	1.5	(26–29)	—	1	—	1	1
II	1.0	(15–25)	—	3	1	1	—
III	3.0 per week	(14–26)	—	2	—	—	—

Persistence of Hearing Loss

Impairment of hearing due to ototoxic drugs such as dihydrostreptomycin is usually per-


GROUP I
KM 0.5 gm/day 59-61 gm total
GROUP I
KM 1 gm/day 112-163 gm total
GROUP I
KM 1.5 gm/day 143-149 gm total
GROUP II
KM 1 gm/day 39-120 gm total
GROUP III
KM 3 gm/week 37-54 gm total

FIGS. 1–5. Formalized audiograms showing various degrees of hearing loss. Initial hearing level for each ear indicated by dot, final hearing level by arrow point. Total dose of kanamycin in grams shown for each patient.

manent. Reports of reversible losses have been few. The hearing loss from kanamycin was permanent in all but 2 of the present patients. In patient *A. C.*, the usual type of abrupt perceptive loss developed at 8,000 cycles per second after 52 gm. of kanamycin. However, a lesser flat perceptive loss involving all of the other frequencies appeared at the same time. This did not occur in other patients, and five weeks after the kanamycin was stopped the hearing at frequencies below 8,000 cycles per second returned to normal. In the second patient (*P. A.*), a bilateral perceptive loss involving frequencies above 1,000 cycles per second was present before treatment. The patient also had a bilateral tinnitus. The hearing impairment and tinnitus had developed following previous dihydrostreptomycin therapy. During treatment with kanamycin, the tinnitus became worse and the hearing loss increased. However, when the treatment was stopped, the hearing gradually improved, reaching almost the pretreatment level at the end of three months.

Tinnitus

In *Group I*, 8 of the 12 patients showing hearing loss also reported tinnitus. One patient (*P. A.*), whose tinnitus was already present before the

kanamycin treatment was begun, reported that it became worse during treatment. Another patient (*E. M.*) noticed tinnitus more than two weeks after she had first reported that her hearing was impaired. In all of the other patients, the tinnitus preceded or accompanied the onset of hearing loss. Of the 5 patients who developed hearing loss in *Group II*, 4 noted tinnitus. Its onset coincided with the appearance of the hearing loss in all of the cases. One of the 2 patients who developed hearing loss in *Group III* reported tinnitus at about the same time as the hearing became impaired.

Four patients reported a definite improvement in the tinnitus within four to eight weeks following discontinuation of kanamycin.

Relation to Nephrotoxicity

Mild to moderate nephrotoxic effects of kanamycin have been observed by almost all clinical investigators. In the present series, granular casts appeared in the urine of all patients receiving a daily dose of kanamycin of 0.5 gm. or more. In several patients a slight transient rise in the blood urea nitrogen was noted during treatment. This disappeared spontaneously, although the drug was continued. It could not be correlated with the onset or incidence of ototoxicity. In only one patient (*C. T.*) did signs of a more severe nephrotoxic effect appear. This patient, who was a diabetic, had a variable degree of albuminuria prior to treatment. The blood urea nitrogen was 18 mg. per 100 ml. The patient's initial audiogram showed a mild, bilateral, mixed type of hearing loss. Following two weeks of therapy (0.5 gm. of kanamycin twice daily; 15 mg. per kg. daily), the blood urea nitrogen was 28 mg. per 100 ml. During the sixth week of treatment, the patient's hearing began to deteriorate rapidly. He had received a total of 40 gm. of kanamycin at this time. The blood urea nitrogen now was 82 mg. per 100 ml. Plasma concentrations of kanamycin must also have been very high but, unfortunately, were not measured. Although the drug was stopped immediately, the hearing became progressively worse over the following three weeks, and both ears finally became totally deaf. Several days following the onset of the hearing loss, the patient also developed a severe disturbance of equilibrium. This finding is discussed further below.

A similar case was seen after treatment on another service. This man was not a tuberculous patient, but had a neurogenic bladder as a result of a spinal fracture, and had developed a urinary tract infection. He had received 1 gm. of kanamycin daily for seven days, and then 4 gm. daily for nine days, making a total of 43 gm. During this time he also received 6 gm. of streptomycin as well as chloramphenicol, penicillin, and sulfonamide for short periods. Two days after kanamycin was stopped, hearing difficulty was first noted. By the fifth day he was totally deaf and has remained so. The blood urea nitrogen was not determined at the time this occurred. When it was done six weeks later, it was normal.

Vestibular Effects

There were several patients who showed abnormal responses to caloric stimulation of the labyrinths prior to treatment. It was believed that this was due to previous streptomycin therapy. Patient *C. T.*, who showed severe nephrotoxicity, was the only one who exhibited disturbance of equilibrium as a result of kanamycin treatment. Several days following the onset of hearing loss, his gait became ataxic. The condition became so severe that he was unable to walk without assistance. Caloric and rotation tests did not reveal any loss of function of the semicircular canals, and neurologic findings were normal. The patient gradually recovered his equilibrium and he was able to walk quite well four months after kanamycin was stopped.

Discussion

In this series of patients the incidence of ototoxicity was closely related to the total amount of the drug administered. Approximately one-third of the patients on daily therapy had developed hearing loss after 60 gm. of kanamycin, and more than two-thirds after 120 gm. (table 2).

No correlation was found between the incidence of ototoxicity and the average daily dose in milligrams per kilogram of body weight, as Finegold has reported (6, 16, 17). Since the number of patients involved in the study was small and the range of dosage was limited, it is not proper to draw a conclusion in this regard.

Age is another important factor in the incidence of hearing loss following the administration of ototoxic drugs. Perceptive loss of hearing after the fifth decade is very common, and it may be assumed that in older patients the cochlea is

more susceptible to the toxic effects of drugs. The present data are not adequate to test this assumption, since only 2 of the patients were older than fifty years.

No clear indication has been obtained that patients with hearing loss due to dihydrostreptomycin or streptomycin are more susceptible to the ototoxic effect of kanamycin, but this possibility should be kept in mind, especially since even a moderate loss superimposed upon a loss already present may seriously impair the patient's ability to understand speech. The possibility also exists that kanamycin may sensitize the patient to the ototoxic effects of subsequent treatment with streptomycin or dihydrostreptomycin. One patient in *Group I* who showed no impairment of hearing after 59 gm. of kanamycin developed a loss at 8,000 cycles per second two months later, after receiving 1 gm. of streptomycin daily for only thirty days.

Finegold has found a definite correlation between renal insufficiency and the incidence of ototoxicity (6, 16, 17). The present experience does not permit a conclusion on this point, as there were only 2 patients in whom the question of renal insufficiency arose. One had a definite impairment of renal function, while the other had a severe urinary tract infection. It should be noted that these were the only 2 patients in whom the ototoxic effects of kanamycin progressed even after administration of the drug was stopped, and who became totally deaf. Several instances of severe or total loss of hearing in patients with renal insufficiency have been reported by other writers (9–15).

Although, in the series of patients studied, there was a correlation between the total amount of the drug administered and the incidence of ototoxicity, a wide variation was found in the amount of kanamycin which produced ototoxic effects in individual patients. This ranged from 32 gm. for patient *P. A.* to 134 gm. for patient *N. W.* It is impossible, therefore, to predict when ototoxicity will develop in any one patient. Although several instances have been reported in the literature in which hearing loss developed after amounts of kanamycin smaller than 32 gm., all have occurred in patients with impaired renal function. From the information available, kanamycin, in dosage not exceeding 1 gm. daily (15 to 25 mg. per kg. of body weight), may be given with reasonable safety up to a total dose of 20 to 25 gm. to patients in the younger age group if they have no evidence of renal disease. So that the onset of ototoxicity may be detected, all patients who receive kanamycin should have weekly hearing tests and should be instructed to report tinnitus at once if it occurs. If the kanamycin is stopped as soon as tinnitus or hearing loss first appears, the hearing is not likely to deteriorate further—except in cases with renal insufficiency.

Because of the many reports of severe hearing loss, and in some instances vestibular damage, which have followed small amounts of kanamycin given to patients with renal insufficiency, it is believed that kanamycin should not be given to such patients, unless as a life-saving measure. If it is administered to patients with any evidence of renal disease, the blood urea nitrogen should be determined frequently and, if it shows a sudden or progressive rise, kanamycin should be discontinued. If the drug is not stopped until the first evidence of ototoxicity appears, it may be too late to prevent a rapid progression of hearing loss to a state of total deafness.

Comparison with Other Antimicrobial Drugs

While no attempt has been made in this study to compare the ototoxicity of kanamycin directly with that of streptomycin and dihydrostreptomycin, it is instructive to compare the results of treatment with 1.0 gm. of kanamycin daily with those obtained by Mahady and co-workers (20) in a much more extensive study using similar doses of streptomycin and dihydrostreptomycin (table 3). It is apparent that the cochlear effects of kanamycin are much more frequent, and the vestibular effects much less frequent, than those of the streptomycin series.

An attempt to rank the five ototoxic drugs, streptomycin, dihydrostreptomycin, viomycin,

TABLE 3

OTOTOXICITY OF KANAMYCIN, STREPTOMYCIN, AND DIHYDROSTREPTOMYCIN

Drug (1 gm./day for 120 days)	Vestibular	Auditory
Kanamycin (15)*	1 (7%)	13 (87%)
Streptomycin (82)†	25 (30%)	2 (2.5%)
Dihydrostreptomycin (84)†	9 (11%)	12 (14%)

* Numbers in parentheses indicate number of patients under treatment.

† From Mahady and associates (20).

TABLE 4
TOXICITY RATINGS OF VARIOUS ANTITUBERCULOUS DRUGS

Drug	Vestibular	Auditory	Renal
Streptomycin	+++	+	—
Dihydrostreptomycin	+	++	—
Viomycin	+?	+++?	++?
Neomycin	±	++++	++++
Kanamycin	±	+++	++

— = No toxicity
± = Rare
+ = Slight
++ = Moderate
+++ = Severe
++++ = Very severe
? = Insufficient data

neomycin, and kanamycin, in terms of their vestibular, cochlear, and renal toxicities, is shown in table 4. The findings of Mahady and associates mentioned above are used as a basis of comparison for streptomycin and dihydrostreptomycin. Kanamycin differs from the streptomycin series in its greater cochlear and renal toxicity and in its lower incidence of vestibular toxicity.

Because of the close chemical and antimicrobial similarity between kanamycin and neomycin, particular importance attaches to the comparison of their toxic effects. The studies of Carr and his co-workers (21) and Waisbren and Spink (22) provide a basis for the evaluation of neomycin toxicity. In Carr's series, four of six patients who received neomycin (0.25 to 1.0 gm. every twelve hours) became partially deaf after four to six weeks, with a progressive loss of hearing that became almost complete even though treatment was stopped immediately. Waisbren and Spink reported deafness in five of their sixty-three cases treated with 1.5 to 2 gm. of neomycin daily for only four to sixteen days, the earliest audiometric changes or subjective complaints of difficulty in hearing occurring after seven and nine days. Four of these five patients had severe intrinsic renal disease, and the fifth had recurrent pyelonephritis.

Although these data are not sufficient for a definitive comparison between kanamycin and neomycin, it is clear that the two drugs closely resemble each other in their ototoxic and nephrotoxic effects. For a particular dose level, however, hearing impairment may occur somewhat sooner with neomycin than with kanamycin. In experimental animals, neomycin has shown a greater degree of cochlear toxicity than has kanamycin (18).

In the early clinical reports, viomycin was said to show both vestibular and cochlear toxicity. Incidence of impairment of hearing at high frequencies was estimated at 20 to 30 per cent. Three cases of sudden total deafness occurred among some 200 patients treated at various hospitals. The degree of toxicity appeared to vary considerably from one lot of viomycin to another (23). It is not clear that kanamycin shows any important advantage over the best lots of viomycin so far as cochlear toxicity is concerned.

SUMMARY

Hearing loss was the most frequent manifestation of ototoxicity in patients on long-term kanamycin therapy. It was detected after total doses ranging from 32 to 134 gm. in 17 of 21 patients who received 0.5 to 1.5 gm. daily, and after 37 to 54 gm. in 2 of 12 patients who received 3 gm. weekly.

The incidence of ototoxicity was closely related to the total amount of the kanamycin administered. Hearing loss occurred first in the higher frequencies in all cases.

Withdrawal of kanamycin resulted in cessation of progression of the hearing loss in all but one patient, who had impaired renal function. He became totally deaf. Two patients showed partial recovery of hearing during the weeks following withdrawal of the drug. In all of the other patients, the hearing remained at the level at which it was when kanamycin was stopped.

Tinnitus was present in 13 of the 19 patients who showed a hearing loss, and preceded or coincided with the appearance of the loss in all but one case. It diminished in 4 patients following cessation of kanamycin treatment. Tinnitus was absent in patients who did not show a hearing loss.

One patient developed ataxia. He recovered during the following four months. The incidence of hearing loss in patients on long-term kanamycin treatment was greater than that reported in patients who had received similar dosage of streptomycin and dihydrostreptomycin.

Attention is called to the greatly increased probability of serious hearing impairment when kanamycin is given to patients with renal insufficiency. Kanamycin closely resembles neo-

mycin in ototoxicity and nephrotoxicity, just as it does in chemical structure and antimicrobial activity.

Sumario

Kanamicina. II. Ototoxicidad

La pérdida de audición fué la manifestación más frecuente de la ototoxicidad en los enfermos que recibieron terapéutica a largo plazo con kanamicina. Se descubrió la misma después de recibir dosis totales que variaron de 32 a 134 gm. en 17 de 21 enfermos que recibieron de 0.5 a 1.5 mg. diarios y después de 37 a 54 gm. en 2 de 12 enfermos que recibieron 3 gm. senales.

La incidencia de la ototoxicidad se relacionó íntimamente con la cantidad total de kanamicina administrada. En todos los casos, la pérdida auditiva ocurrió primero con las frecuencias más altas.

La supresión de la kanamicina hizo cesar la agravación de la pérdida auditiva en todos los enfermos, menos uno, que padecía de insuficiencia renal. Este quedó absolutamente sordo. Dos enfermos mostraron recuperación parcial de la audición durante las semanas consecutivas a la supresión de la droga. En todos los demás enfermos, la audición permaneció como estaba al suspenderse la kanamicina.

Hubo zumbidos de oídos en 13 de los 19 enfermos que mostraron pérdida auditivo, precediendo o coincidiendo con la aparición de la pérdida en todos los casos, menos uno. Disminuyeron los mismos en 4 enfermos a continuación del cese de la kanamicinoterapia. No se observó zumbido de oídos en los enfermos que no revelaron pérdida de audición.

Un enfermo manifestó ataxia, reponiéndose durante los cuatro meses siguientes. La incidencia de pérdida auditiva en los enfermos que recibieron kanamicinoterapia a largo plazo fué mayor que la descrita en enfermos que habían recibido dosis semejantes de estreptomicina y dihidrostreptomicina.

Llámase la atención sobre las probabilidades mucho mayores de que sobrevenga grave insuficiencia auditiva cuando se administra kanamicina a sujetos que padecen de insuficiencia renal. La kanamicina se parece sumamente a la neomicina en su ototoxicidad y nefrotoxicidad, como sucede también con su composición química y actividad antimicrobiana.

Resume

Kanamycine. II. Ototoxicité

La perte de l'ouïe fut la manifestation la plus fréquente chez les malades soumis à une thérapeutique à long terme à base de kanamycine. Cela fut trouvé après des doses totales allant de 32 à 134 gm, chez 17 de 21 malades qui reçurent 0,5 à 1,5 mg. par jour, et après 37 à 54 gm, chez 2 de 12 malades qui reçurent 3 gm par semaine.

La fréquence de l'ototoxicité fut en rapport étroit avec la quantité totale de kanamycine administrée. La perte de l'ouïe se produisit d'abord pour les hautes fréquences dans tous les cas.

L'arrêt de kanamycine eut comme résultat de faire cesser la progression de la perte de l'ouïe, chez tous sauf un, qui avait une fonction rénale altérée. Il devint complètement sourd. Deux malades montrèrent une récupération partielle de l'ouïe pendant les semaines qui suivirent l'arrêt du médicament. Chez tous les autres malades, l'ouïe resta au niveau où elle était quand on arrêta la kanamycine.

Des bourdonnements furent présents chez 13 des 19 malades qui présentèrent une perte de l'ouïe, et furent précédés ou coïncidèrent avec le début du déficit, chez tous sauf un. Ils diminuèrent chez 4 malades après l'arrêt du traitement par la kanamycine. Il n'y eut pas de bourdonnements chez les malades qui ne présentèrent pas de perte de l'ouïe.

Un malade devint ataxique. Il récupéra pendant les quatre mois suivants. La fréquence de la perte de l'ouïe chez les malades soumis à un long traitement à la kanamycine fut plus grande que celle que l'on a signalée chez les malades ayant reçu des doses semblables de streptomycine et de dihydrostreptomycine.

Les auteurs attirent l'attention sur la plus grande probabilité d'une atteinte sérieuse de l'ouïe, lorsque la kanamycine est prescrite à des malades ayant une insuffisance rénale. La kanamycine ressemble étroitement à la néomycine par son ototoxicité et sa néphrotoxicité de la même façon que ces produits se ressemblent par leur structure chimique et leur activité antimicrobienne.

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THE INFLUENCE OF THE NUMBER OF BACILLI ON THE DEVELOPMENT OF TUBERCULOUS DISEASE IN CHILDREN[1]

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Introduction

Since 1949 all child contacts of patients with pulmonary tuberculosis in the East Suffolk and Ipswich area have been examined by tuberculin studies and chest roentgenography. In 1954 the results of these examinations were reviewed in relation to the bacteriologic classification of the index cases (1) at the time of diagnosis (2). At that time publication of surveys of child contacts in this country were relatively few and included only one post-war survey conducted in a large city (3).

In table 1 the results of certain of the contact surveys made in England since 1931 may be seen. There were a number of surveys available from other countries. In most of these, contacts of index cases with tubercle bacilli in the sputum were compared with the contacts of sputum-"negative" index cases, the sputum being examined by direct microscopic techniques. These surveys, therefore, compared contacts of known infectious cases with the contacts of cases believed to be noninfectious (4–8).

By 1949 patients with pulmonary tuberculosis whose sputum was negative for tubercle bacilli by direct microscopy were having their sputum cultured for tubercle bacilli. Therefore, it is now possible in this paper to compare three groups of contacts:

1. Contacts of index cases whose sputum was positive for tubercle bacilli on direct microscopy
2. Contacts of index cases whose sputum was negative by direct microscopy, but contained tubercle bacilli as determined by culture
3. Contacts of index cases whose sputum was negative for tubercle bacilli on culture.

There is evidence (9–11) that sputum in which tubercle bacilli are found by direct microscopy contain many more bacilli than sputum in which tubercle bacilli can be discovered only after culture. Therefore, it is now possible to compare the effect on child contacts of large doses of tubercle bacilli with smaller doses of bacilli.

There are two recent surveys concerning this problem (12, 13), the results of which are discussed later.

There is considerable epidemiologic evidence that exposure to a high dosage of tubercle bacilli has some influence on the development of disease in a contact. However, experimental work suggests that all pulmonary tuberculosis originates from a small dose of infecting bacilli and that the subsequent development of disease then depends on the natural and acquired immunity of the contact.

This paper records further clinical evidence concerning this problem. The results collected up to the end of 1953 have been published previously (2). These results have been reviewed and, in addition, the material collected between 1954–1956 has been included.

Observations

Method and Materials

Index cases: This investigation was designed to discover the relationship between the sputum state of the index case at the time of diagnosis and the condition of the children in the household as revealed by tuberculin tests and chest roentgenography. By definition, an index case is the first case of pulmonary tuberculosis known in any household (1).

All patients aged fifteen or older registered as cases of pulmonary tuberculosis at the Ipswich & East Suffolk Chest Clinic between January 1, 1949, and December 31, 1956, were considered for inclusion. Two cases of undoubted tuberculosis in children less than fifteen were also included.

The criteria for inclusion were a recent chest roentgenogram and sputum test; the patient also must have been living in a normal household; and attempts must be made to examine the contacts. These criteria and subsequent exclusions are shown in table 2.

Some of the cases registered during the period

[1] From St. Helen's Hospital, Foxhall Road, Ipswich, England.

TABLE 1

CONTACT SURVEYS IN GREAT BRITAIN

Reference	Year of Testing	Test	Age	Tuberculin-positive Contacts of Patients with Sputum Positive for Tubercle Bacilli on Direct Microscopy	Tuberculin-positive Contacts of Patients with Culture Positive for Tubercle Bacilli on Culture	Tuberculin-positive Contacts of Patients with Sputum Negative for Tubercle Bacilli on Direct Microscopy
			years			
Dow and Lloyd (70)	1931	Mantoux, 10 TU, 100 TU	0–15	75.5% of 184	Sputum not cultured	36.8% of 95
D'Arcy Hart (71)	1932	Mantoux, 1 TU	0–10	69% of 42	Sputum not cultured	42% of 26
Aspin and Lotinga (3)	1948–1949	Mantoux, 10 TU	0–14	66% of 332	Sputum not cultured	34% of 108 Tuberculin-positive contacts of culture-negative cases
Shaw and Wynn-Williams (12)	1950–1953	Jelly, 100 TU	0–15	65.2% of 374	26.8% of 228	17.6% of 221
van Zwanenberg (2)	1949–1953	Jelly, 100 TU	0–15	72.9% of 225	45.8% of 96	38.4% of 99
Loudon, Williamson, and Johnson (72)	1954–1955	Heaf	0–14	46.9% of 226	30.1% of 196	17.6% of 347

TABLE 2

Criterion for Inclusion of Index Case	Number of Exclusions	Reason for Exclusion
Recent chest roentgenogram	10	No chest film. Diagnosed *in extremis*.
Sputum test or laryngeal swab	9	No sputum. Diagnosed *in extremis*.
	9	No sputum. Diagnosed after death.
Must live in "normal" household	34	Lived in institutions.
Attempt made to examine contact	6	Private patients of doctors who did not wish contacts examined.

of investigation failed to satisfy the definition of an index case because previous cases of pulmonary tuberculosis were known in their households. Thirty households were excluded because of this.

In addition, in twenty-six households more than one case of adult pulmonary tuberculosis was diagnosed during the period under review. By definition, the first case diagnosed in the household was included and the case diagnosed later was excluded. In theory, this may have created some bias in the results, and it is discussed later.

The index cases included in the surveys were classified on the results of sputum examinations, the sputum being examined for tubercle bacilli at the time of diagnosis. Only sputum examinations made within one month of diagnosis were used for this classification.

It was the routine practice to examine the sputum of all patients diagnosed, or suspected, as suffering from pulmonary tuberculosis. The direct Ziehl-Neelsen technique was first used. If this proved to be negative, then the sputum was cultured for tubercle bacilli by a modified Petroff technique (14). If the patient had no sputum, laryngeal swabs were taken and cultured for tubercle bacilli, using an alkaline technique (14). Index cases are classified as:

C. Cases = Sputum positive for tubercle bacilli by Ziehl-Neelsen technique.

B. Cases = Sputum negative for tubercle bacilli by Ziehl-Neelsen technique, but positive on culture

A. Cases = No sputum. Laryngeal swabs positive for tubercle bacilli on culture

O. Cases = Negative for tubercle bacilli, even on culture.

Contacts: All those aged fifteen or less living in the household of the index case at the time of diagnosis were included as contacts. Until January, 1954, the routine testing of contacts consisted of:

Tuberculin jelly test (15).

If negative to jelly test, then Mantoux test, 100 TU.

If positive to either of these tests, a chest film was obtained and repeated every three months for a year.

If normal on both tests, BCG vaccination was offered after a chest roentgenogram had been obtained and found to be normal.

Those who were negative to both tuberculin tests and also refused BCG vaccination were offered further tuberculin testing at three-month intervals.

The tuberculin reactions of contacts up to a year after the first tuberculin test, or until BCG vaccination, were included in the results.

In nearly every case, contact between the index case and the child was broken soon after diagnosis. Any infection from the index case was therefore likely to cause conversion of the tuberculin state or a visible roentgenographic abnormality within a few months. Any similar change occurring a year later was more likely to be due to infection from another source.

During 1953 it became apparent that the tuberculin tests in use were not satisfactory. The jelly test, when used with flourpaper, gave an appreciable number of false positive results, i.e., positive to the jelly test but negative to other tuberculin tests. Other workers confirmed the unreliability of the flourpaper jelly test (16, 17).

The 100 TU Mantoux test was also said to give a significant proportion of reactions that were "non-specific for tuberculous infection" (18).

The obvious unreliability of the flourpaper jelly test upset a fundamental part of the investigation. Early in 1954 this test was abandoned and, following some preliminary trials, the Heaf multiple-puncture test was used as the only tuberculin test for routine contact testing (19).

There have been various reports on the reliability and accuracy of the Heaf multiple-puncture test (20–25). Recently, in a fully planned trial, its reliability in comparison with the Mantoux test has been demonstrated (26).

Since January, 1954, all contacts have been tested by the multiple-puncture tuberculin test, using PPD (Weybridge).

Steps have been taken to try to confirm all of the tuberculin jelly tests of the children tested before 1954. As a result of this retesting, the earlier tuberculin results can be divided into three groups:

Group 1: those subjects originally tested with the jelly test and found to be jelly negative. These children, with few exceptions noted in the results, were retested with 100 TU Mantoux at the time as a preliminary to BCG vaccination. There is, therefore, confirmation of these results of the jelly test.

Group 2: those subjects originally tested with the jelly test who had positive reactions and also developed some roentgenographic abnormalities in the lung fields. In many of these it was thought to be unjustifiable to retest if calcification had developed in the lesion. The calcification was accepted as confirmation of tuberculous infection as, in Great Britain, "almost all calcified foci in the lung are tuberculous in origin" (27).

The definition of calcification was that used by Shaw and Wynn-Williams (28), "Calcification was diagnosed when an opacity of uneven density was present or when an opacity was seen to develop in the area of a pulmonary lesion." As in the Danish Tuberculin Index (29), care was taken to include only definite calcification. The Danish experience was that, in reading adult chest roentgenograms, vascular shadows and calcification in costal cartilages led to most errors. Costal cartilages did not give rise to any difficulty in reading the children's films in the present survey. Vascular shadows, particularly near the hilum of the lung, can give difficulty. These vascular shadows have a smooth and definite outline and are of even density, whereas calcification in a tuberculous focus is irregular and of uneven density. The study of serial roentgenograms helps to resolve these difficulties, but when there was any doubt the shadow was not regarded as calcification.

In reviewing the chest films, 5 children were found to have large calcified lesions in the lungs at the time when the index case was diagnosed. It appeared that these children must have been infected at least some months previously and, in theory, might have been infected by another index case. Their number was too small to affect the issues discussed, and they are included. Four children were contacts of *C.* cases and one of an *O.* case.

Group 3: those subjects found originally to be tuberculin reactors to the jelly test who had no roentgenographic abnormality. Most of these were later retested with other tuberculin techniques. If the subsequent tuberculin reaction was negative, then that test was accepted and the previous jelly test was regarded as false. These are included as negative reactions in the results.

Theoretically, some of these subjects could have had true tuberculin reactions to the jelly test and then reverted naturally before they were retested. This is considered in the section on assessment of the method.

It is theoretically possible that, in cases in which positive tuberculin jelly test reactions were later confirmed by another test, the original jelly test gave a false positive reaction, and the child converted naturally before the second test was applied. In the unlikely event of this coincidence, it would not upset the significance of the results.

Results

Of 341 *C.* index cases, 305 child contacts were examined. Of these contacts, 200 (65.6 per cent) were tuberculin positive and 71 had pulmonary lesions which later showed calcification. Four of these children have since developed post-primary tuberculosis.

Of 171 *A.* and *B.* index cases, 136 child contacts were examined. Thirty-five (25.7 per cent) of these contacts were tuberculin positive, but only 2 had pulmonary lesions which later showed calcification. None of them have developed post-primary tuberculosis.

Of 227 *O.* index cases, 167 child contacts were examined. Thirty-five (21.0 per cent) of these were tuberculin positive and 4 had pulmonary lesions which later showed calcification. None of these children have developed post-primary tuberculosis.

Assessment of the Method

Tuberculin test: The original test used was unsatisfactory, but its results have been confirmed when possible. In a number of cases, it was not possible to confirm or refute these results. The unconfirmed results with the jelly test consisted of 31 positive and 8 negative contacts of *C.* cases, 8 positive and 2 negative contacts of *A.* and *B.* cases, and 6 positive and 3 negative contacts of *O.* cases. If all of these were wrong, then the percentage of tuberculin-positive reactors would read as in table 3, which does not upset the significance of the results. It is unlikely that all of these unconfirmed tests were false. A possible estimate of their value can be obtained from the results of retesting on the rest of the children in the *C.* group who had positive reactions to the jelly test.

One hundred and sixteen children originally tested by the jelly test and found to be positive were retested very soon after by the Heaf test, and 17 were found to be negative. This would mean that the unconfirmed reactions to the jelly tests would be 15 per cent too much. The true figures, therefore, lie considerably nearer to those given as the results than to those given in table 3. As already mentioned, there is considerable evidence to show that the multiple-puncture test is reliable and accurate. Owing to variations in technique, it may be misleading to compare the results of tuberculin tests performed at different clinics.

TABLE 3

MAXIMAL ERROR DUE TO UNCONFIRMED TUBERCULIN JELLY RESULTS

	As Recorded	Upper Limit	Lower Limit
	per cent		
C. Cases	65.6	68.2	55.9
A. and *B.* Cases	25.7	27.2	19.9
O. Cases	21.0	22.8	17.4

Influence of bovine tuberculosis: During the period of this survey a number of children in East Suffolk are known to have been infected by bovine tuberculosis. It is assumed in the present study that the tuberculin-positive children have been infected by the index cases. This assumption is probably reasonable provided other sources of infection can be excluded. Precautions have been taken in the selection of index cases to exclude other human sources of infection. No means could be devised to exclude the possibility of bovine tuberculous infection in the milk.

Half the population served by the Chest Clinic lives in Ipswich, and the other half lives in East Suffolk. Ipswich has had "safe" milk (pasteurized or T.T.[2]) for some years. In East Suffolk measures to make the milk safe have been introduced in recent years, but even now they are still incomplete. If bovine tuberculosis was responsible for a significant number of these children being tuberculin positive, then one would expect this to be apparent when comparing the percentage of tuberculin-positive child contacts of Ipswich and East Suffolk.

In the three groups *C.*, *A.* and *B.*, and *O.*, the percentage of tuberculin-positive children in Ipswich was 59.6, 23.3, and 13.6, respectively, whereas in East Suffolk it was 67.6, 18.9, and 22.3. This is shown in tabular form in table 4. Only confirmed tuberculin tests were used. Five children had lived for an appreciable time in both Ipswich and East Suffolk, and they are shown separately.

During the period under review, 11,454 children were tuberculin tested routinely in school surveys. The percentage of these children who were tuberculin positive was 7.5 for those living in Ipswich and 19 for those living in East Suffolk. This is also shown in table 4.

The difference between the percentage for the contact children, either taken individually or as a series, is not statistically significant. It seems unlikely, therefore, that bovine tuberculosis caused a significant number of the tuberculin-positive results in the child contacts.

[2] T.T. (tuberculin-tested) milk is from herds that are tuberculin tested every six months, and it is bottled on the farm.

TABLE 4

PERCENTAGE OF TUBERCULIN TESTS THAT WERE POSITIVE IN CHILD CONTACTS OF INDEX CASES, *C.*, *A.* and *B.*, AND *O.*, LIVING IN IPSWICH OR EAST SUFFOLK. ALSO RESULTS OF TUBERCULIN TESTING NONCONTACT CHILDREN IN THESE AREAS

	C.	*A.* and *B.*	*O.*	Noncontact Children
Ipswich	59.6% of 156	23.3% of 73	13.6% of 81	7.5% of 8,435
East Suffolk	67.6% of 106	18.9% of 53	22.3% of 76	19% of 3,019

Number of tuberculin-positive children excluded because they lived in both areas: 5(4 × *C.* cases and 1 × *O.* case)

Diagnosis of index cases: The discovery of tubercle bacilli in the sputum or laryngeal swab of a patient with abnormal shadows on the chest roentgenogram is usually accepted as sufficient evidence for the diagnosis of pulmonary tuberculosis. In the *C.* and *A.* and *B.* cases this "proof" of the diagnosis was available. In the *O.* cases there has been no bacteriologic proof of the diagnosis. Every case has now been followed for more than a year, and most of the patients have been treated in the hospital. In no case has the diagnosis been altered or even seriously doubted.

The disease of 3 of the *O.* cases has relapsed during follow-up observation, producing sputum positive for tubercle bacilli on direct microscopy. They and their contacts are included as they were at the time of the original diagnosis (as *O.* cases).

Diagnosis of disease in the child contacts: One of the difficulties in the diagnosis of tuberculous disease in children is to define what constitutes disease. There are physicians who maintain that a positive tuberculin test, if it is truly specific, denotes that the child has suffered an active infection and is therefore diseased, even when the lesion cannot be localized and causes no symptoms. Myers is probably the greatest exponent of this view. There are many other definitions of tuberculous disease in children (13).

Whatever the definition may be, there is obviously a difference between the tuberculin-positive child with no localizable lesion, and the tuberculin-positive child with a definite tuberculous lesion seen on a chest roentgenogram. In this paper, therefore, those with definitely diagnosed tuberculous lesions seen on a chest film are defined as having tuberculous disease. The diagnosis of pulmonary tuberculosis in children is often difficult to confirm by bacteriologic methods. Because of this, there is no bacteriologic proof of the diagnosis in these children. This could cause serious error in the results.

The findings of an abnormal pulmonary shadow in a tuberculin-positive child may be diagnosed as pulmonary tuberculosis whatever its cause may be. It seemed advisable, therefore, to have some confirmation of a diagnosis of pulmonary tuberculosis in the children. It was decided to accept the appearance of calcification in a lesion as confirmation of the diagnosis.

Not all significant childhood pulmonary tuberculosis results in calcification (30), and some calcification may not be recognized on the roentgenogram (31). It is likely, therefore, that the figure accepted here as showing significant pulmonary disease is too low. Certainly there were 4 tuberculin-positive contacts who developed abnormal roentgenographic shadows lasting more than a month which later disappeared, leaving an apparently normal film. In the absence of visible calcification, these have not been accepted as confirmed cases of pulmonary tuberculosis.

Of the 71 children in the *C.* group who were diagnosed as having pulmonary disease, 38 had lesions which were thought to be serious enough for the children to be confined to bed for treatment, and a further group had visible abnormal shadows on their first roentgenograms.

In reviewing the chest films, a number of children were found to have roentgenographically visible lesions proceeding to calcification which were not recognized as abnormal when the child's first film was seen. In some, the original lesions were small; in others they were obscured by shadows of normal structures; and in others, the lesions were quite visible but were not recognized as abnormal until calcification was seen.

Age of the children: In spite of the care taken to include only one index case for each contact, there can be no proof that the tuberculin-positive

TABLE 5

DISTRIBUTION OF CHILD CONTACTS IN AGE GROUPS

Index Cases	*C.*	*A.* and *B.*	*O.*
Number of children	305	136	167
Per cent of group aged:			
0–5	43	49	40
6–10	32	25	30
11–15	25	26	30

children were in fact infected by the corresponding index case. It is quite possible that some of the children were infected by other sources.

Children less than five years old are unlikely to have been infected outside the home. Tattersall and his colleagues showed that the source of infection of tuberculin-positive school entrants could usually be found at home (32, 33).

Some of the older children may have been infected before their contact with the index case or before the index case contracted the disease. There is no certain way of discovering whether this happened. However, there is no reason why one group of contacts should be affected more than another, provided that the groups contain similar child populations and that there is not a preponderance of older children in any one group.

In the *C.* group, 43 per cent of the children were 0–5 years old; 32 per cent, 6–10; and 25 per cent, 11–15. The figures for the *A.* and *B.* groups were 49, 25, and 26 per cent; and for the *O.* group, 40, 30, and 30 per cent. This is shown in table 5.

It may be noted that in each case the largest group is in the 5-year-old and younger children. The *O.* group had the highest percentage of older children, although it had the lowest percentage of tuberculin-positive child contacts.

The groups were also comparable in respect to the number of contacts examined per index case, the number of contacts refusing examination, and the age distribution of the index cases.

DISCUSSION

The index cases with sputum positive for tubercle bacilli on direct microscopy infected more than twice as many of their contacts as did the *A.* and *B.* cases (65.6 to 25.7), but they caused recognizable tuberculous disease in more than fifteen times as many (23 to 1.5). The disproportion between infection rates and disease rates in these two groups is statistically significant.

Both groups (*C.*, and *A.* and *B.*) of index cases were comparable in most other respects. They all suffered from pulmonary tuberculosis proved by positive bacteriologic findings. They all lived in household contact with the children concerned. The reasons for their selection were similar. They differed, however, in one respect. The *C.* cases had sputum positive for tubercle bacilli by direct microscopy, whereas the *A.* and *B.* cases were positive on culture only.

There is evidence that the difference between sputum positive for tubercle bacilli by direct microscopy and that positive by culture only is entirely related to the number of tubercle bacilli present. Corper and Cohn (10) estimated that sputum positive on direct microscopy by Ziehl-Neelsen technique contained more than 100,000 bacilli per ml., whereas cultures could be positive when sputum contained 10 to 100 bacilli per ml. This also was the finding of Pottenger (9). More recently, Cruickshank (11) estimated that direct microscopic methods were likely to fail if the concentration of tubercle bacilli was below 50,000 per ml., whereas culture was likely to be "several hundred times more sensitive."

Although it cannot be claimed that the method used has definitely classified individual patients according to the number of bacilli they were excreting, it is likely that this is true of the groups of patients. Thus the patients in the *C.* group were excreting many more bacilli per day than were patients in the *A.* and *B.* groups.

Provided other factors are constant, it seems possible that the number of bacilli present in the sputum of index cases may be responsible for the size of the tuberculous lesion in the contact. The difference between the tuberculin-positive child with a normal roentgenogram and the tuberculin-positive child with tuberculous disease diagnosed by a shadow in the chest film is probably due to the size of the tuberculous process in the lung.

Assuming that both groups of children were infected by inhaling tubercle bacilli, then there would be pathologic lesions in the lungs, but whether such lesions would give roentgenographic shadows that could be seen would depend on a number of factors, e.g., the size of the lesion, the position of the lesion, the technical quality of the chest film, and the skill of the physicians reading the chest film.

In this survey, the quality of the roentgenograms and the skill of the physicians have been more or less constant. There seems to be no reason why children infected by *C.* cases should develop lesions in different areas of the lung than children infected by *A.* and *B.* cases, although there is no evidence on this point. The size of the lesion remains as the principal deciding factor in the diagnosis of tuberculous disease in these children.

Obviously, the development of tuberculous disease cannot be due to one factor alone. So far, the dosage of infection has been considered without regard to some of the other factors which might themselves explain the results being discussed. It is advisable to exclude the possibility that a fallacy in selection could have caused these results. Although the groups discussed are selected, there seems to be no reason why the selection should have caused bias in one group more than in another. It will be remembered that, when two cases of adult pulmonary tuberculosis were diagnosed in a household during the survey, the first case was included as the index case. This first case, however, may not be the source of infection in the household; the other patient may have had the disease longer and more severely. In this way, it is possible that households included in cases *O.*, *A.*, or *B.* should have been more correctly assigned to *C.*

There were twenty-six such households altogether and, if they were excluded altogether, the remaining figures would still retain the same significance.

It is also necessary to consider the time factor. If other factors were constant, it seems reasonable to suppose that the longer the period of contact, the more likely it is that a child will become infected. It is also probably true that, as a group, *C.* cases were infectious for a longer period before diagnosis than the *A.* and *B.* groups. This may explain why *C.* contacts have a higher tuberculin-positive rate than the other contacts. It will not explain the disproportion between infection rates and disease rates unless repeated infections are the cause of the roentgenographically visible disease.

This idea of repeated infection's playing a part in the development of primary disease has been put by Macpherson (34) among others. She states:

> Those children who are in contact with heavily infected active cases and are exposed to repeated doses of infected material, and who live with the tuberculous person in poor accommodation, tend to develop lesions which are liable to give rise to recognisable clinical and radiological change.

Assuming that repeated infections occur, what is the pathologic mechanism whereby primary tuberculous lesions finally result? These children have not previously had primary lesions so this is not superinfection as usually defined. Repeated infections do not apparently give rise to multiple primary lesions, since these are comparatively rare. Rich (35) will not accept the idea that repeated infections might lower resistance since he says "there is nothing in immunology to support it."

The present paper can offer no direct evidence on this point. All that can be said is that those children who were exposed to the largest doses of bacilli and were probably repeatedly infected showed considerably more disease than might be expected when compared with other children exposed to less infection.

A further possibility on which this paper can throw no light is that the childrens' susceptibility to infection may vary and at some periods they become more likely to develop disease. Those children in contact with infectious cases over long periods might then be more likely to develop manifest tuberculous lesions than children in contact with infectious cases for only a short time. There is experimental evidence that animals can become temporarily more susceptible to various infections following the injection of certain biologic preparations (36, 37).

Familial susceptibility is another factor that may possibly play a part. In the great majority of cases there was a direct blood relationship between the child contact and the corresponding index case. If it is true that certain families are more susceptible to tuberculous disease, then the findings reported here might possibly be explained on the basis of inherited susceptibility.

There are undoubtedly families that seem to be abnormally susceptible to the disease. Clarke (38) records two such families, and van Zwanenberg (39) described a family in which both parents and nine children all developed post-primary tuberculosis.

Severe circumscribed epidemics of this type are not confined to families alone, and inheritance may not, therefore, be the most important factor. There is the well-known epidemic reported by Hyge (40). Other epidemics in schools are found

in a paper by the Joint Tuberculosis Council (41). Protheroe (42) recently reported an epidemic in a military band.

Pearson (43), in a well-known statistical study on tuberculosis among the children of sanatorium patients, concluded that there was a diathesis for pulmonary tuberculosis which was inherited. This work was severely criticized by Rich, with regard to both the methods employed and the conclusions. As Rich points out, even if it is accepted that the offspring of tuberculous parents develop tuberculosis more frequently than the offspring of nontuberculous parents, there is nothing in Pearson's work that demonstrates that heredity plays a greater role in the development of tuberculosis than does intimate exposure to a source of infection.

Pearson later tried to prove his hypothesis (44) by comparing the frequency with which nontuberculous marital partners contract tuberculosis from their tuberculous mates with the frequency with which children contract disease from tuberculous parents. He assumed that both the children and the mates were exposed to similar infection.

Since the children more commonly developed disease, he concluded that this was due to inheritance of tuberculous susceptibility from the tuberculous parent. This work is open to many criticisms. The children are obviously of a different age group than the mates of tuberculous patients and, furthermore, it is likely that most of the children had not previously been infected, whereas many of the adults must have been. Therefore, tuberculin-negative children were being compared with tuberculin-positive adults. This criticism applies equally to other studies in which adults and children are compared (45).

Puffer (45) found that, until the age of thirty, the attack rate in contacts of sputum-positive patients was significantly higher than in the contacts of sputum-negative patients. After a thirty-year follow-up study, she reported that these attack rates became reversed and, after a forty-year follow-up, "the cumulative probability of developing tuberculosis at different ages" for children of index cases was the same whether the index case was sputum positive or negative. She cites this as evidence of familial susceptibility. Unfortunately, she does not quote the number of persons followed or the number who were lost sight of during the forty years; nor is there any evidence of whether they encountered further infectious tuberculosis.

In a recent paper by her colleagues (46), they stress that in a follow-up study of twenty-two years the contacts of sputum-positive patients had a disease rate of 3.3 per 100 observation years, compared with 1.8 per 100 for the contacts of sputum-negative cases, which seems to contradict some of Puffer's earlier conclusions.

It has been shown that children living in households with open tuberculosis show significantly more disease when they are closely related to the source of infection than when they are distantly related or unrelated (47, 48).

The criticism of this evidence of familial susceptibility is that it is obvious that the contact between patients and their own children is far more intimate than between patients and somebody else's children.

Clarke (38) points out that tuberculosis frequently occurs in persons without any family history. He estimates that two thirds of cases diagnosed with tuberculosis have no such history. Aspin (49) in a study of 680 index cases with pulmonary tuberculosis found that in 73 per cent of the cases there was neither previous nor subsequent tuberculosis in the family. Therefore, increased familial susceptibility to tuberculosis can hardly account for a large proportion of patients who develop the disease.

Probably the most convincing evidence of familial susceptibility comes from twin studies. Diehl and Verschuer (50), Kallman and Reisner (51), Uehlinger and Kunsch (52) have all produced figures to show that, when one twin is tuberculous, significantly more of the co-twins develop tuberculosis if the twins are monozygous.

Berghaus' work did not confirm this (quoted by Clarke, 38). Other criticisms can also be made. First, that twins are "abnormal," and, therefore, conclusions from twin studies may not apply to the general population. Second, great care must be taken to include all of the twins with tuberculosis. Since monozygous twins look alike, these will be discovered more easily than dizygous twins, and this bias may be shown in the results. Other difficulties in this type of study have been reported by Simonds (53).

Therefore, although there is some evidence in favor of familial susceptibility as an element in the development of tuberculous disease, except

in certain rare families, this does not seem to be the most important factor.

In the present survey it is difficult to see how familial susceptibility can be blamed as a major cause for a patient's developing sputum positive for tubercle bacilli on direct microscopy. Many factors are involved in the development of disease of this degree of severity.

The length of time between the beginning of the activity of the disease and the moment of diagnosis must play a principal part. It cannot be doubted that, if many of the patients of the *C.* cases had been diagnosed earlier, they would not have had infectious sputum on direct microscopy. It is known that the disease in several of the *O.* cases relapsed later on and the sputum became infectious on direct microscopy. It therefore seems highly unlikely that inheritance can play the major role in deciding the sputum status of patients. The association of disease in child contacts with sputum positive for tubercle bacilli on direct microscopy in the index case is, therefore, not likely to be due directly to familial susceptibility to the disease.

The next factor to be considered is that of virulence of the bacilli. If the virulence of tubercle bacilli for human beings varies, then it is possible that the more virulent the bacilli, the more likely they will be to cause disease that will develop sufficiently to give sputum positive for tubercle bacilli on direct microscopy. Such virulent bacilli would also be more likely to cause disease in the child contacts.

This study can offer no evidence on this point. Investigations into virulence by other workers have not shown any correlation between the clinical severity or household spread of tuberculosis and the virulence of tubercle bacilli in isolation (54–56).

Finally, malnutrition has always been considered a factor of importance in the development of tuberculous disease. However, since the last war, malnutrition, other than in rare isolated cases, has ceased to exist in Ipswich and East Suffolk. It cannot have played any significant part in the development of these cases of tuberculosis. These various factors, therefore, do not seem to explain the high disease rate in the *C.* group. It is now necessary to discuss briefly other evidence in favor of the hypothesis that the number of bacilli in an infectious dose influences the development of disease.

There is some evidence from the few surveys that index cases have been classified as positive for tubercle bacilli on direct microscopy or on culture, or as negative. In the two papers available, the methods used were not quite the same as in this paper, and the results cannot be compared strictly with the results of this study. However, they do show similar trends.

Shaw and Wynn-Williams (12), in their survey, used the simple jelly test and roentgenography on their contacts. If their results are expressed according to the present classification, the results are:

Group	Number of Children	Per Cent Tuberculin Positive	Per Cent with Disease
C.	374	65.2	12.8
A. and *B.*	228	26.8	2.6

Hertzberg, in a large monograph (13) described results, using the Pirquet test and fluoroscopy of his contacts. His selection of cases was rather less strict than in this study, and his classification of tuberculous disease was different. Again, applying the present classification to his figures:

Group	Number of Children	Per Cent Tuberculin Positive	Per Cent with Disease
C.	1,387	68.3	29.2
A. and *B.*	1,393	33	6.3

In both of these surveys, the infection rate in the *C.* groups was two to three times the rate of that in *A.* and *B.* groups, whereas the disease rate was four to five times as great in the *C.* groups. The disproportion is again statistically significant.

There is evidence also from different types of surveys. Daniels and associates (57), in the first Prophit Survey, found a higher morbidity in nurses and students exposed to greater infection. In particular, primary tuberculous lesions were more often demonstrated in those particularly exposed. Of the nurses who became tuberculin positive while on tuberculosis wards, 4.76 per cent developed recognizable lesions, as opposed to 2.46 per cent of the nurses who became tuberculin positive while on general wards. Thompson (58) reported that tuberculin-positive children with a definite history of contact showed

a higher percentage of calcified lesions than those with no contact history. Madsen, Holm, and Jensen (59) found that 38 per cent of "converters" had roentgenographic lesions if they were particularly exposed to infection, but only 11 per cent of those who were less exposed had such lesions. Frohlick (60) followed 2,429 tuberculin-positive children. The contacts of definite cases had three times the morbidity of the non-contacts. In the experimental field it is known that, when laboratory animals are infected with tubercle bacilli, the severity of the resultant disease is proportional to the dose of infecting bacilli, and this is so whatever the method of infection employed (35, 55).

Conditions that would increase the opportunities for infection with large doses have also been shown to be associated with a higher incidence of tuberculosis. This is particularly true of overcrowding. Often, however, overcrowding is associated with other factors such as malnutrition, poverty, exertion, poor medical care, lack of light, inadequate ventilation, et cetera, so that it is impossible to decide which of these is mainly responsible for the high incidence of the disease.

Hart and Wright (61) showed a correlation between the ratio of persons per room and the percentage of tuberculous families in two areas; also that the incidence of tuberculosis was higher in families overcrowded in small rooms than in families overcrowded in large rooms. Stein (62, 63) reported that "density of dwelling occupation and additional overcrowding together constitute the major factors in the association of the social complex with respiratory tuberculosis in Glasgow."

Not all of the evidence available is in support of the hypothesis that the size of the infecting dose may play a definite part in the development of recognizable disease. The principal evidence against it is based on the work of experimental pathologists, particularly Chausee and Lange. It is interesting to note that, although this experimental work was completed more than thirty years ago, it is still accepted by modern specialists on the subject (55, 64). This study can produce no experimental work of this type, and the present writer is in no position to criticize the techniques involved. Since the conclusions derived from the work are relevant to the problem of the size of the infecting dose, a brief review of the work is necessary. The review is taken almost entirely from a paper by Dahl (65) who summarized and criticized the work of the experimental pathologists.

The experiments can be divided into six groups:

1. When droplets were sprayed so that they could pass down glass tubes bent and tapered to resemble human bronchi, only those of a diameter of 10 to 20 μ or less passed through the tube. All larger droplets were thrown against the walls of the tube, provided that the air flow was similar to that occurring in the air passages of man.
2. Droplets larger than 20μ fell to the floor within a minute. Smaller droplets could be air borne for hours.
3. Animals exposed to a spray of infected droplets showed the same picture of disease, whether exposed immediately or after a period of five minutes.
4. Bacilli in droplets that are thrown against the walls of bronchi do not subsequently produce disease.
5. Small droplets of 10 to 20μ could only contain one or—at most—very few tubercle bacilli.
6. When such small infected droplets were sprayed at an animal, primary foci of disease could be found later, corresponding approximately to the number of the infected droplets.

From this work it is argued that only very small droplets could reach the alveoli and cause disease. If numerous droplets were inhaled, then numerous primary foci should result. In man the primary focus is usually single, although on occasions multiple foci have been recorded (66, 67). If all this is true, then a single primary focus in man should be the result of a single small infected droplet.

This work has been criticized by Rich, who maintains that a droplet of 0.25 mm. in diameter would contain several hundred bacilli. This, however, is considerably larger than the figure accepted as the size of the infecting droplets. Rich's other criticism is that the terminal bronchiole, which has no ciliary mechanism, could be infected instead of the alveoli. The size of the terminal bronchiole seems to be about twice the size of the alveolus. Prime and McMichael (68) estimate the alveolus as 0.08 to 0.13 mm., and Johnston considers 0.2 mm. (69) as the size of the terminal bronchiole. Since terminal bronchioles are approached by the same air passages as alveoli, the evidence that droplets larger than 20μ cannot drift down the air passages seems to exclude the possibility of larger droplets' infecting the terminal bronchiole.

In more recent and extensive experiments on air-borne infection in rabbits, Dr. Lurie and his associates (70) have shown that bacilli in a very fine suspension cause more tubercles to develop in the lung than do bacilli in larger and visible droplets. Tuberculous disease is generated only in the respiratory divisions of the bronchial tree where the smallest particles can penetrate. Also, the number of bacilli estimated as inhaled and the number of tubercles generated closely approximate the actual numbers involved or their multiples.

In conclusion, therefore, there is considerable evidence from the clinical point of view that the size of the infecting dose plays a part in deciding whether a primary tuberculous lesion in the lung of a child will become large enough to be recognized. There is also evidence from experimental work that the size of the infecting droplet cannot be large enough to carry more than a few bacilli, and that multiple primary lesions are uncommon.

These views would seem to be entirely opposed to one another. The clinician may accept the clinical findings in practice, but ignore the experimental work from the laboratory or dismiss it as being inapplicable to man, or valid under experimental conditions only. The experimental pathologist may accept the work done in the laboratory because the conditions were properly controlled, but dismiss clinical studies in which complete control of conditions was impossible. Some selection certainly occurred and the findings can be explained by other factors.

Assuming, however, that both sets of facts are correct, it seems possible to produce an hypothesis that could satisfy both parties. One aspect of infection seems to have been ignored, namely, the factors that decide where an infected droplet shall enter an alveolus and cause disease. It seems quite reasonable that such variable factors as position of the body, phase and depth of respiration, condition of the alveolar epithelium, et cetera, might all play a part. Since alveoli are very small, it is also reasonable to assume that these factors would be similar at any one time for a number of adjacent alveoli. Provided, therefore, that a considerable number of infected droplets in the air were inhaled, those factors that determined that one droplet should infect one alveolus might also cause nearby droplets to infect adjacent alveoli.

In opposition to this, Dahl wrote "It is quite unbelievable that several germs could have been inhaled in different droplets to the same point in the lung and together produce one single primary focus." If chance was the deciding factor for the site of infection, this statement might be true. Since definite physical factors are involved, then, provided there are many infected droplets available, these factors are likely to bring several or many droplets to the same position in the lung.

Summary

All new patients with pulmonary tuberculosis who attended chest clinics in the East Suffolk and Ipswich area between January, 1949, and December, 1956, were classified according to their sputum findings at the time of diagnosis:

(*1*) Three hundred and forty-one index cases, with sputum positive for tubercle bacilli on direct microscopy, had 305 child contacts, 65.6 per cent of whom were tuberculin positive and 23 per cent had tuberculous lesions demonstrated roentgenographically.

(*2*) One hundred and seventy-one index cases, with sputum positive for tubercle bacilli on culture only, had 136 child contacts, 25.7 per cent of whom were tuberculin positive; only 1.5 per cent had tuberculous lesions.

(*3*) Two hundred and twenty-seven index cases, with sputum negative for tubercle bacilli even on culture, had 167 child contacts, 21 per cent of whom were tuberculin positive and 2.4 per cent had tuberculous lesions.

The patients in group (*1*) infected more than twice as many contacts as did patients in group (*2*), but caused recognizable tuberculous lesions in fifteen times as many contacts. This disproportion is statistically significant. The implications of this discovery are discussed with special reference to the influence of the number of bacilli on the development of tuberculous disease in children.

Sumario

Influjo del Número de Bacilos sobre la Aparición de Enfermedad Tuberculosa en los Niños

A todos los enfermos nuevos que tenían tuberculosis pulmonar y que asistían a clínicas tuberculosas en la zona de East Suffolk e Ipswich

entre enero de 1949 y diciembre de 1956, se les clasificó conforme a los hallazgos en el esputo al hacer el diagnóstico:

(*1*) Trescientos cuarenta y un casos del índice, con esputo positivo para bacilos tuberculosos a la microscopía directa, tuvieron 305 contactos con niños, 65.6 por ciento de los cuales eran positivos a la tuberculina y 23 por ciento tenían lesiones tuberculosas observables radiográficamente.

(*2*) Ciento setenta y un casos del índice, con esputo positivo para bacilos tuberculosos únicamente en cultivos, tuvieron 136 contactos con niños, 25.7 por ciento de los cuales eran positivos a la tuberculina; solamente 1.5 por ciento tenían lesiones tuberculosas.

(*3*) Doscientos veintisete casos del índice, con esputo negativo para bacilos tuberculosos, hasta en cultivos, tuvieron 167 contactos con niños, 21 por ciento de los cuales eran positivos a la tuberculina y 2.4 por ciento tenían lesiones tuberculosas.

Los enfermos del grupo (*1*) infectaron más del doble de los contactos que los del grupo (*2*), pero ocasionaron lesiones tuberculosas reconocibles en un número quince veces mayor de contactos. Esta desproporción posee importancia estadística.

Se discuten las connotaciones de este descubrimiento con referencia particular al influjo del número de bacilos sobre la aparición de enfermedad tuberculosa en los niños.

Resume

Influence du nombre de bacilles sur le développement de la maladie tuberculeuse chez les enfants

Tous les malades nouveaux avec une tuberculose pulmonaire dans le Suffolk de l'Est et dans la région d'Ipswich qui vinrent à la consultation pour maladies de la poitrine, entre Janvier, 1949, et Décembre, 1956, furent classés suivant ce qu'on trouva dans leur expectoration au moment du diagnostic:

(*1*) Trois cent quarante et un cas, avec une expectoration positive pour le bacille tuberculeux, à l'examen microscopique direct, comportaient 305 contacts avec des enfants, 65,6% d'entr'eux avaient une cutiréaction positive et 23% avaient des lésions tuberculeuses prouvées par la radiographie.

(*2*) Cent soixante et onze cas, avec expectoration positive pour le bacille tuberculeux, démontrée par culture seulement, eurent 136 contacts avec des enfants, parmi lesquels 25,7% eurent une réaction positive à la tuberculine. Seulement 1,5% avait des lésions tuberculeuses.

(*3*) Deux cent vingt sept cas, avec expectoration négative, même après culture, avaient 167 contacts avec des enfants parmi lesquels 21% avaient une réaction positive à la tuberculine et 2,4% avaient des lésions tuberculeuses.

Les malades du groupe *1* eurent deux fois plus de contacts infectieux que les malades du groupe *2* mais ils causèrent des lésions tuberculeuses reconnaissables quinze fois plus. Cette disproportion est importante au point de vue statistique.

Ce qu'implique cette découverte est discuté en insistant spécialement sur l'influence du nombre de bacilles dans le développement de la tuberculose chez les enfants.

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RESECTION AND CONCOMITANT THORACOPLASTY IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1]

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Introduction

This paper deals with 137 cases of resection and concomitant thoracoplasty in patients operated upon between February, 1953, and August, 1958. During the same period an additional 211 resections were performed in which thoracoplasty was considered unnecessary at the time.

Many thoracic surgeons believe that thoracoplasty, whether performed earlier, concomitantly, or later, in selected cases would offer better protection for patients with pulmonary tuberculosis who have had resection of a lung or of a large upper portion of a lung. This is because of the immediate "space" problem and the later danger of reactivation of residual disease.

Steele carried out a survey among Veterans Administration hospitals regarding the surgical management of pulmonary tuberculosis and included, among other problems, the use of space-reducing procedures. He found that in approximately 50 per cent of hospitals, from which answers were obtained, moderate to frequent use of concomitant thoracoplasty was reported; in others either prior or subsequent thoracoplasties were performed (1). From this study it becomes obvious that many surgeons consider that it is no longer a question of whether a tailoring procedure should or should not be done in a large resection, but when it should be done.

Iverson and associates first published a report on three cases of pneumonectomy for tuberculosis followed by immediate thoracoplasty in 1949, and a report on an additional seven cases in 1950 (2, 3). In the same year, 1950, Samson and co-workers reported seventeen cases of upper lobectomy and nine cases of pneumonectomy combined with concomitant thoracoplasty (4). Conklin and associates added thirty-eight combined resection-thoracoplasties a year later (5). Despite the voluminous publications in the literature dealing with surgical treatment of pulmonary tuberculosis, there has been a scarcity of articles on the subject of combined operations since 1951.

The original program in this Sanatorium was to avoid thoracoplasty for any resection unless it was rendered necessary by postoperative complications. Two hundred and twenty-two resections were managed this way, the first pulmonary resection dating back to February, 1940. It was believed, however, that the over-all results could be improved. The first concomitant thoracoplasty in a pneumonectomy case was done in February, 1953. This was the only case in which a planned second-stage thoracoplasty was subsequently carried out. Since then, concomitant thoracoplasties have been practiced in cases in which there would be a large residual space following resection. It has not yet been found necessary to postpone the thoracoplasty to a later date on account of the condition of the patient. Very occasionally, in seriously ill patients, a prior Semb's operation has been done. In these cases it is thought preferable to wait for a few months to see the results of the thoracoplasty alone before performing a resection if that is still considered advisable. The latter group of advanced cases is small and is not included in this paper.

Materials and Methods

Using the lateral decubitus position, the rhomboids and the lower fibers of the trapezius are incised close to their vertebral attachment in order to save the nerve and blood supply. The latissimus dorsi and serratus anterior are incised as distally as practicable so as to spare much of the function of these muscles. The chest is usually entered through the fifth rib bed. A short posterior segment of 2 to 3 inches of the fourth rib is frequently taken out to facilitate exposure, thus obviating too forceful spreading of the ribs resulting in dislocation of the costotransverse joints, fracture of the ribs, and excessive soft tissue damage. In pneumonectomies, a concomitant thoracoplasty is performed unless the condition of the patient at the time contraindicates such an added procedure. It takes fifteen to twenty minutes longer. In other major upper resections a thoracoplasty is performed if the inflated lung

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cannot come close to filling up the pleural cavity and particularly when the remaining lung is adherent to the chest wall. It is believed that it often takes just as much time to free the lung, with no assurance that it will obliterate the pleural space, as to do a small tailoring thoracoplasty. Should an empyema develop when the latter procedure has been adopted, it will be a localized one instead of the total variety.

When a thoracoplasty is decided upon, approximately two-thirds of the third rib is removed. The origin of the pectoralis minor is not disturbed. The second rib is usually transected at the costochondral junction. The first rib is as frequently resected as not. It is removed particularly if it is desirable to relax the contralateral apical disease. In the writer's experience, the first rib contributes little in preventing scoliosis. The posterior segments of the ribs are cut usually 1 cm. from the tip of the transverse processes, thus entailing minimal detachment of the paravertebral muscles. It is not necessary to remove the transverse processes in tailoring and in Semb's type of thoracoplasty, as there is no lung underneath the decostalized chest wall to prevent it from falling against the mediastinum. The stout costotransverse ligaments, the most important elements in maintaining a straight posture, are thus left intact. Without them the side is too weak to counterbalance the power of the tissues of the contralateral side. As pointed out previously, if the transverse processes are removed in multistaged thoracoplasties, it will result in some scoliosis no matter how conscientiously the physiotherapy is carried out (6). Theoretically, at least, the intact costotransverse joints will retain the function of the intercostal muscles. The reduced pulmonary function from frozen costotransverse joints in Marie-Strumpell's disease is too well known to deserve any comment.

Only a short posterior segment of the fourth rib is resected lest the inferior angle of the scapula slip forward and cause great discomfort and elevation of the shoulder and, hence, scoliosis.

When more than a five-rib thoracoplasty is deemed necessary, a short piece of the sixth and the seventh ribs should be removed. If the seventh rib is intact, the scapula nearly always slips forward and produces the aforementioned undesirable effect. In pneumonectomy cases, six to nine ribs are removed. A complete thoracoplasty is not necessary. When only a six-rib thoracoplasty is performed the first rib is left untouched. It is inadvisable and unnecessary to remove long anterior segments from the third rib downward as that will decostalize the cardiac region and defunctionalize the pectoralis minor. It serves no useful purpose. It produces deformity and added morbidity.

The soft tissues of the chest wall are carefully approximated on closure of the incision. The integrity of the muscles is better preserved than if they were assaulted twice or more by multistaged operations. A bun is strapped to the armpit and an elastic binder designed by the writer is then applied to go around the opposite shoulder and axilla to prevent paradoxical movement.

The patients may lose considerable blood from the intercostal drainage tube postoperatively, as the diseased lung is frequently removed extrapleurally, which results in free communication between the pleural and the subscapular spaces. The intercostal bundles cannot be fully approximated without compromising the desired collapse. They may require a greater amount of blood replacement. The impression is that these patients as a rule need more analgesics than patients with resection alone. There should be more liberal use of narcotics so that they can be encouraged to bring up sputum or blood. Finally, intermittent positive pressure breathing is helpful in preventing atelectasis.

Observations

Analysis of clinical data: The data tabulated in tables 1–8 are fairly clear. The following points, however, are stressed: It may be seen in table 1 that 74 per cent of the patients in this series had far advanced disease as compared with 36.5 per cent of all of the patients who have had 570 resections performed since 1940. It may also be noted in table 2 that 71.6 per cent of this series had contralateral disease.

TABLE 1

Classification of Diseases and Types of Operation

Classification of Disease	137 Patients Who Had Combined Operation February, 1953–August, 1958	570 Lung Resections February, 1940–August, 1958
	per cent	*per cent*
Far advanced	74	36.5
Moderately advanced	24	58.4
Minimal	2	5.1

TABLE 2

Breakdown of Diagnoses In 137 Patients with Combined Operation

	Number of Cases	Per Cent
Ipsilateral		
Far advanced	94	68.5
Moderately advanced	41	30.0
Minimal	2	1.5
Contralateral		
Far advanced	12	8.8
Moderately advanced	36	26.3
Minimal	50	36.5
No disease	39	28.4

TABLE 3
INDICATIONS FOR OPERATION

	Number of Cases	Per Cent
Open cavity with sputum positive for tubercle bacilli (within 3 months preoperatively)	54	39.4
Open cavity with sputum negative for tubercle bacilli (within 3 months preoperatively	64	46.7
Residual disease, et cetera	19	13.9

TABLE 4
ASSOCIATED DISEASES IN 137 PATIENTS WITH COMBINED OPERATION

	Number of Cases	Per Cent
Alcoholism	9	6.6
Mental illness	7	5.1
Empyema with or without bronchopleural fistula	5	3.6
Resection failures	4	2.9
Contralateral frozen lung or thoracoplasty	3	2.2
Diabetes	3	2.2
Bronchial asthma	2	1.5
Severe arthritis	2	1.5
Severe hypertension	1	0.7
Carcinoma of lung	1	0.7

TABLE 5
OPERATIONAL RISKS
(Age, Extent of Disease, Status of Sputum, Drug Resistance, Cardiopulmonary Function, Associated Disease, et cetera)

	Number of Cases	Per Cent
Good risk	14	10.2
Average risk	70	51.1
Poor risk	53	38.7

TABLE 6
TYPES OF OPERATION

	Number of Cases	Per Cent
Lobectomy	58	42.2
Lobectomy plus	21	15.3
Pneumonectomy	13	9.5
Segmentectomy	45	33.0

The period of average preoperative drug treatment was 17.3 months. Thirty per cent of the patients were re-treatment (drug) cases. The incidence of drug resistance was high. There were 83 males and 54 females. (The males outnumbered the females 3 to one during the last three years of this series.) The average age was 36 years, the youngest patient being 16 and the oldest, 73. Twenty-six per cent of the patients were Indians and Eskimos.

Indications for operation (table 3): Open cavities and sputum positive for tubercle bacilli were noted in 54 cases (39.4 per cent); 19 patients (13.9) had no cavities and noninfectious sputum. As is the practice of others, it has been the writer's method to resect solid lesions greater than 2 cm. in diameter. Fifty-three patients (38.7 per cent) were classified as poor risks (table 5). This method of grouping is necessarily arbitrary. Ninety-two (67 per cent) of the patients had lobectomy, lobectomy plus, or pneumonectomy (table 6). The majority of the segmentectomies were bi- or trisegmentectomies. One hundred and three patients had four- to five-rib thoracoplasties and only one patient had nine ribs resected (figure 1). These thoracoplasties were all done concomitantly with the resections. The operation was performed on the right in 71 patients and on the left in 66 patients.

It is interesting to note that only 2 patients developed empyema as a postoperative complication; both empyemas were tuberculous and the organisms were drug resistant (table 7). In one patient, a large cavity was inadvertently rup-

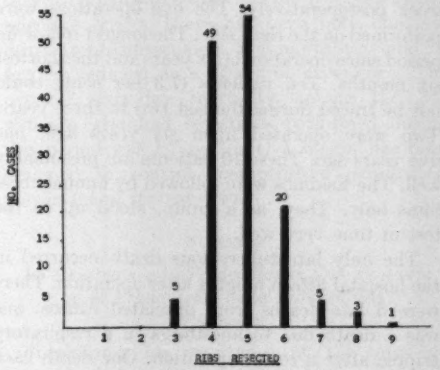

FIG. 1. Number of ribs resected in 137 patients with combined operation.

TABLE 7
POSTOPERATIVE COMPLICATIONS

	Number of Cases	Per Cent
Atelectasis	7	5.1
Persistent air space	5	3.6
Bronchopleural fistula and empyema	2	1.5
Spread of disease	2	1.5
Clotted hemothorax	1	0.7
Operative sacrifice of a lobe	1	0.7
Pulmonary edema	1	0.7
Water intoxication	1	0.7
Deaths (within 2 months postoperatively)	5	3.6

TABLE 8
LATE RESULTS OF 137 PATIENTS TRACED FOR PERIODS FROM SIX MONTHS TO TWO YEARS

	Patients	Per Cent
Early postoperative death (included in table 7)	5	3.6
Late tuberculous death	1	0.7
Loss to follow-up	10	7.3
Alive with active disease	2	1.5
Alive with inactive disease	114	83.2
Deaths from unrelated causes	5	3.6

tured during resection; the other had tuberculous bronchitis not visible by bronchoscopy. The latter patient is still being treated.

Of the 5 postoperative deaths, 2 were definitely preventable, 3 were due to error in judgment and overconfidence. All occurred within the first week postoperatively. The five operations were performed on the right side. The longest follow-up period since operation is six years and the shortest six months. Ten patients (7.3 per cent) could not be traced during the last two to three years. Two were operated upon six years ago, one five years ago. These 10 patients are presumably well. The Eskimos were followed by annual chest films only. They, as a group, stood up to the test of time very well.

The only late tuberculous death occurred in the hospital fifteen months after operation. There were 5 late deaths from unrelated causes: one was a death due to anesthesia in a respiratory cripple after a rectal operation. One death each occurred by drowning, suicide, car accident, and carcinoma of the colon, respectively (table 8).

DISCUSSION

Prior to 1953, about 10 per cent of the resections in the Mountain Sanatorium were done for thoracoplasty failures and approximately another 10 per cent of the patients underwent postresection thoracoplasty out of necessity. The latter group frequently required multiple procedures. Thus, this series of 137 patients who had combined operations among 348 resections (39 per cent) is not as large as it appears. The present rate is around 30 per cent.

The question of impairment of pulmonary function following thoracoplasty is closely related to the presence or absence of scoliosis (4). None of the patients in this series had more than a minimal degree of scoliosis even when it was present. There was also an absence of deformity in the average case. The slight sinking of the upper anterior chest wall was hidden by the pectoralis major.

In most major resections without thoracoplasty, the intrapleural pressure is rendered highly negative in an effort to obliterate the pleural space. The patients experience severe pain. They cannot take a deep breath and they are prone to develop atelectasis and spread of disease. Air spaces and fluid formation constitute ever remaining troublesome problems. These may lead to empyema and bronchopleural fistula. The complications often develop before a postresection thoracoplasty can be carried out even if it were planned. Morbidity and mortality rates are high (7, 8). It would be better to perform a tailoring thoracoplasty in anticipation of these possible difficulties at the time of the resection. There were only 2 patients in the present series who developed empyema.

Marked mediastinal shift following a major resection without a thoracoplasty in the older age group will produce an increase in residual volume (9). This can be avoided by an early thoracoplasty.

When the diaphragm is elevated in major resections without a space-reducing procedure, subjectively, there is less freedom of respiratory movement. The breath is cut short during the early phase of inspiration while the opposite hemidiaphragm continues to descend. The lower part of the lung is pulled up to fill the large residual space. This newly placed lung is put in a position of disadvantage. The ribs hinge on the costotransverse joints. There is little air exchange

in the apical and posterior segmental region since these segments are closest to the fulcrum of the bony thorax and farthest away from the diaphragm. Incidently, the writer advances this as a theory to explain why tuberculosis commonly affects these two segments.

In patients with recently active contralateral disease or scattered ipsilateral disease, it would be considered unwise to allow the diseased lung to overexpand after resection without a space-reducing procedure, contrary to the long-established principle of collapse therapy. Many of these patients could be offered the benefit of a combined operation if there were no other contraindication. Only 4 patients in this series developed late reactivation of tuberculosis during this period of study. One of them died of his disease, one is still under treatment, the third and the fourth patients have been discharged home, well after further treatment.

A prior Semb's type of thoracoplasty, like postresection thoracoplasty, means another major operation, an arduous experience for the patient, to say the least. However, this operation has been performed in an occasional patient with very poor pulmonary function, tuberculous bronchitis, a copious amount of infectious sputum, et cetera. In these cases, five or six months' delay is preferable in order to see what thoracoplasty alone can do before performing resection.

A postresection thoracoplasty will collapse the upper portion of the expanded lung which has become adherent to the apex of the pleural cavity and thereby further reduces the pulmonary function. This operation was performed only once.

Experience has convinced the writer that a concomitant thoracoplasty can be done safely in most cases of tuberculosis at the time of a major resection in order to reduce the incidence of postoperative complications and later reactivations of residual disease, particularly in cases of bilateral, extensive lesions.

The combined procedure is no doubt a more serious operation than resection alone. The patients should be carefully assessed before operation and they require intensive postoperative care. The surgeon should see them frequently. The use of a simple chest binder with a bun strapped to the armpit is considered invaluable in stabilizing the chest wall over the decostalized area. There is little difficulty with paradoxical breathing. The writer has also performed 51 one-stage primary thoracoplasties, removing five to nine ribs during the same period. He considers drug resistance as an indication for resection rather than as a contraindication. This view is not shared by Hirdes and associates in their series of pneumonectomies without thoracoplasty (9).

No attempt has been made to compare the results here with those of others. Some of the patients were definitely calculated risks. The sixty-day postoperative mortality of 3.6 per cent in this selective series is acceptable. The over-all mortality of 1.7 per cent among 348 resections during the period between February, 1953, and August, 1958, compares favorably with most published series. By the frequent use of the combined operation, the sphere can be widened in the selection of cases of bilateral disease in which resection is considered mandatory. The clinicians are more willing to recommend—and the patients to accept—a single operation than multi-staged procedures. A combined operation is not as traumatic as some might think.

Summary

Results of 137 cases of pulmonary resection and concomitant thoracoplasty among a total of 348 resections in the treatment of tuberculosis are presented. The operations were carried out between February, 1953, and August, 1958. The follow-up period is between six months and six years. Ninety per cent of the patients were followed for more than one year.

Concomitant thoracoplasty is considered a safe procedure when indicated. The sixty-day postoperative mortality rate was 3.6 per cent in this selective series. There has been only one late death from tuberculosis. The mortality could be further reduced as a result of experience. The over-all sixty-day mortality among the 348 patients who had resections performed during this period was 1.7 per cent. A binder has been designed to control postoperative paradoxical movement of the chest wall.

Sumario

La Resección y la Toracoplastia Concomitante en el Tratamiento de la Tuberculosis Pulmonar

Preséntanse los resultados de 137 casos de resección pulmonar con toracoplastia concomitante entre un total de 348 resecciones ejecutadas

en el tratamiento de la tuberculosis pulmonar. Estas operaciones se llevaron a cabo entre febrero de 1953 y agosto de 1958. El período de observación subsiguiente quedó entre seis meses y seis años. A 90 por ciento de los enfermos se les mantuvo en observación más de un año.

Se considera que la toracoplastia concomitante es un procedimiento inocuo cuando se halla indicada. En esta serie selectiva, el coeficiente de mortalidad operatoria de sesenta días fué de 3.6 por ciento. Sólo ha habido una muerte tardía de tuberculosis. Como fruto de la experiencia podría reducirse aun más la mortalidad. La mortalidad global de sesenta días entre los 348 enfermos a quienes se hicieron resecciones durante dicho período fué de 1.7 por ciento. Se ha diseñado una faja que cohibe el movimiento paradójico postoperatorio de la pared torácica.

Resume

Resection et thoracoplastie concomittentes dans le traitement de la tuberculose pulmonaire

L'auteur présente les résultats de 137 cas de résection pulmonaires et de thoracoplastie pratiquées en même temps dans un total de 348 résections effectuées comme traitement de tuberculose pulmonaire. Ces opérations furent pratiquées entre Février 1953 et Août 1958. La période pendant laquelle les malades furent suivis fut de six mois à six ans. Quatre vingt dix pour cent des malades furent suivis plus d'un an.

La thoracoplastie associée est considérée comme un moyen sur, si elle est indiquée. Le taux de mortalité soixante jours après l'opération fut de 3,6% dans la série de malades choisie. Il n'y eut qu'une mort tardive de tuberculose. La mortalité pourrait encore être réduite avec plus d'expérience. La mortalité pendant les soixante jours parmi les 348 malades ayant subi la résection fut de 1,7%. Un bandage a été appliqué pour contrôler les mouvements postopératoires paradoxaux de la paroi thoracique.

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CHEMOTHERAPY IN RESECTION FOR PULMONARY TUBERCULOSIS[1, 2]

Problems of Extensive Disease and Drug-resistant Organisms

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INTRODUCTION

The present report is an analysis of the value of chemotherapy in resection for pulmonary tuberculosis. Results obtained in cases of optimal chemotherapy have been compared with those obtained in cases of poor or marginal chemotherapeutic control. Emphasis has been placed upon the management of patients having extensive disease and (or) drug-resistant organisms.

It is the patients having extensive disease and those having strains of tubercle bacilli resistant to the major antituberculous agents who challenge chemotherapeutic skills, particularly when surgical treatment may prove to be definitive if sufficient chemotherapeutic effect can be provided: first, to achieve a status which will permit surgical intervention and, second, to cover the postoperative period.

MATERIAL AND METHODS

All patients subjected to pulmonary resection at the Chicago State Tuberculosis Sanitarium during a period of four and one-half years are included in this study (table 1). Of the total number of 358 patients, 112 patients had extensive resections, including 75 pneumonectomies, 20 resections of all of one lung except the basal segments, and 17 bilateral resections.

Many patients had previously received antituberculous chemotherapy, usually for long periods with interruptions and a variety of regimens. A total of 100 patients (29 per cent) had problems of chemotherapy as shown in table 2. It is important to note that the figures in the last column of table 2 represent the number and per cent of patients in each category who had major bacterial resistance, sputum positive for tubercle bacilli at the time of resection, or both conditions, each patient being counted only once.

Nearly half of the patients for whom extensive resection was necessary also had problems of chemotherapy.

[1] From the Chicago State Tuberculosis Sanitarium, Chicago, Illinois.

[2] Presented before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

Chemotherapy Regimens

The chief regimens used in the treatment of patients subjected to pulmonary resection are summarized in table 3. Each case is counted only once, being included in the regimen which furnished the chief chemotherapeutic effect, particularly in relation to surgical intervention.

A total of 98 patients received one or more of the so-called minor antituberculous agents. The choice of agents and the timing of their administration were based on the kind and degree of bacterial resistance *in vitro* and the therapeutic goal to be achieved. When bilateral resection was planned, some of the available chemotherapeutic effect was reserved for the second operation.

Streptomycin, PAS, and isoniazid: When the patient's strain of tubercle bacilli appeared to be predominantly susceptible *in vitro* to streptomycin, PAS, and isoniazid, these agents were used in combination throughout the patient's treatment, including the postoperative period.

The sodium salt of PAS was the form of this drug most often prescribed and the usual dose was 4 gm. three times a day.

Streptomycin generally was prescribed in a dose of 1 gm. twice a week except during the immediate postoperative period when daily administration was used, usually for two weeks.

In most cases isoniazid was prescribed in a dose of 100 mg. three times a day. When studies *in vitro* revealed an appreciable degree of resistance to this drug, the total dose was increased to 600 or 900 mg. per day, with the addition of pyridoxine in a dose of 100 to 300 mg. per day. High doses of isoniazid were used also when bacteriologic conversion had not occurred after three months of treatment with isoniazid in the usual dose in combination with streptomycin and PAS.

Viomycin: In cases of apparently complete resistance to streptomycin, viomycin was usually substituted at the time of resection, being prescribed in an amount of 1 to 2 gm. per day administered in one or two doses. In cases of less marked streptomycin resistance, viomycin was usually added to the patient's regimen at the time of surgery, each antimicrobial being given in a dose of 1 gm. per day for the first two weeks of the postoperative period; thereafter, twice a week.

With this regimen the toxicity of viomycin was negligible. In a few cases in which daily treatment with viomycin was continued for more than two or three weeks, toxic effects as previously described were observed, namely nephrotoxicity,

electrolyte disturbances, and damage to the eighth cranial nerve (1, 2).

Viomycin and pyrazinamide: In cases of marked bacterial resistance *in vitro* to both of the major antituberculous agents, streptomycin and isoniazid, pyrazinamide was given in addition to viomycin during the postoperative period. In a few cases, viomycin and pyrazinamide were the only agents used during and following surgery, but more often they were prescribed along with any other drug which might offer a measure of protection.

Pyrazinamide was prescribed in a dose of 30 to 35 mg. per kg. of body weight, the total amount being divided into three doses per day. With this dosage of 2 to 2.5 gm. per day, there was no evidence of hepatitis (3) or of other serious toxicity. Some patients received the combination of viomycin and pyrazinamide for as long as eighteen months.

Since the suppressive effect of viomycin and pyrazinamide is limited in duration as well as in degree, their administration was carefully timed in relation to surgery. In a few cases viomycin was given for several weeks before surgery in order to achieve a specific goal, such as the healing of endobronchial disease. In all other cases, viomycin, or viomycin and pyrazinamide, were prescribed on—or a few days before—the date of resection.

Cycloserine in high dosage: In cases of extensive, bilateral, often progressive disease, the patients received cycloserine in large doses for a number of months or until sufficient improvement had occurred to make surgical intervention feasible. The

TABLE 1

PATIENTS SUBJECTED TO PULMONARY RESECTION
February, 1954–July, 1958

Extensive resection	
Pneumonectomy	75
Resection of lung less basal segments	20
Bilateral resection	17*
Total	112 (31.3%)
Other resections	246
Total	358

* One other patient, included in pneumonectomies, had bilateral resection.

TABLE 2

PROBLEMS OF CHEMOTHERAPY

Procedure	Number of Patients	Previous Chemotherapy	Major Resistance*	Sputum Positive for Tubercle Bacilli†	Resistance and (or) Sputum Positive for Tubercle Bacilli‡
Pneumonectomy	75	43	29	26	41 (54.6%)
Resection of lung less basal segments	20	10	8	7	9 (45%)
Bilateral resection	17	2	2	2	2 (11.8%)
Extensive resections	112	55	39	35	52 (46.4%)
Other resections	246	50	32	24	48 (19.5%)
Total patients	358	105	71	59	100 (29%)

* 4+ growth on 10 γ of streptomycin and (or) 5 γ of isoniazid

† Sputum positive for tubercle bacilli by concentrate at time of resection

‡ Total number and per cent of patients who had problems of bacterial resistance and (or) concentrates positive for tubercle bacilli at time of resection

TABLE 3

CHEMOTHERAPY OF PATIENTS SUBJECTED TO RESECTION*

Chemotherapy	Pneumonectomy	One Lung less Basal Segments	Bilateral Resection	Other Resections	Total
Streptomycin, PAS, isoniazid	41 (12)	12 (2)	9	198 (14)	260 (28)
Viomycin added at surgery	6 (4)	4 (3)	1	20 (11)	31 (18)
Viomycin + pyrazinamide	15 (12)	4 (4)	5 (1)	24 (20)	48 (37)
Cycloserine before surgery	13 (13)	0	2 (1)	4 (3)	19 (17)
Total	75 (41)	20 (9)	17 (2)	246 (48)	358 (100)

* Figures in parentheses denote resistance to streptomycin and (or) isoniazid and (or) sputum positive for tubercle bacilli at time of resection.

antimicrobial was prescribed in a dose of approximately 30 mg. per kg. of body weight, the total amount of 1 to 2.5 gm. being divided into three doses per day. Half of the patients received 2 gm. or more per day.

With a dosage of this magnitude, considerable toxicity for the central nervous system occurred, but in most cases this could be prevented or controlled by the use of pyridoxine, anticonvulsants, sedatives, and tranquilizing agents.

In the group of patients treated with cycloserine and subsequently subjected to resection, additional agents which might increase the patient's chemotherapeutic protection were prescribed at the time of surgery. Viomycin and pyrazinamide were reserved for this purpose when the patient had not received these agents previously. If the patient had tolerated cycloserine well, this drug was usually resumed after surgery.

Other expedients: In order to provide all possible chemotherapeutic effect at the time of resection, variations of the above regimens were used in a number of cases, particularly when the patient had already received all available antituberculous agents and his strain of tubercle bacilli was virtually completely drug resistant.

One such expedient consisted of the administration of five drugs simultaneously—streptomycin, PAS, isoniazid, viomycin, and pyrazinamide—in the hope that the total suppressive effect would be sufficient for surgical coverage. A few patients received cycloserine in addition to these five drugs. As a rule, however, cycloserine in large doses was not administered to patients whose regimen included isoniazid because of a possible additive toxic effect of these drugs upon the central nervous system.

In some cases the therapeutic efficacy of PAS was increased by substituting intravenous for oral administration. The lyophilized sodium salt of PAS was given in a dose of 15 gm. per day for at least two weeks after resection.

Because of evidence that oxytetracycline may have some antituberculous effect when administered in a dose of at least 2 gm. per day (4), this drug was included in the regimen of several patients who were especially marginal from the standpoint of chemotherapeutic protection.

Observations

Surgical mortality: Six (1.7 per cent) of the 358 patients subjected to pulmonary resection died within the postoperative period of sixty days or less. The cause of death was gastrointestinal hemorrhage in 2 cases; post-thoracotomy bleeding in one case; pulmonary infarction in one case; cardiorespiratory insufficiency in one case; and uremia in one case.

Postoperative tuberculous complications: Tuberculous complications encountered in the present series of cases included: persistence of sputum concentrates and (or) cultures positive for tubercle bacilli following resection, subsequent bacteriologic relapse, roentgenographic evidence of increased disease, and tuberculous empyema. Nontuberculous complications are not being presented because they are not relevant to the subject of chemotherapy in resections for pulmonary tuberculosis.

The term postoperative has been stretched to cover the entire period of time between surgery and the date of most recent evaluation and, therefore, both early and late complications are included in the data presented.

Postoperative complications are related to the extent of resection in table 4 and to the principal regimens of chemotherapy in table 5. In both tables the figures in the last two columns represent the number and per cent of patients

TABLE 4

Postoperative Tuberculous Complications

Procedure	Patients*	Surgical Deaths	Sputum Positive for Tubercle Bacilli Postoperatively	Increased Disease	Tuberculous Empyema	Patients with Tuberculous Complications†	Complications‡
							per cent
Pneumonectomy	75 (41)	2 (0)	4 (4)	4 (4)	0	4 (4)	5.5
One lung less basal segments	20 (9)	0	1 (1)	0	0	1 (1)	5.0
Bilateral resection	17 (2)	0	1 (1)	0	1 (1)	1 (1)	5.9
Other resections	246 (48)	4 (3)	11 (8)	6 (6)	4 (2)	13 (10)	5.4
Total	358 (100)	6 (3)	17 (14)	10 (10)	5 (3)	19 (16)	5.4

* Figures in parentheses denote resistance to streptomycin and (or) isoniazid and (or) sputum positive for tubercle bacilli at the time of resection.

† Total number who had one or more tuberculous complications.

‡ Per cent of patients surviving surgery who had tuberculous complications.

in each category who had tuberculous complications of any kind, each patient being counted only once. Again it is important to note that the figures in parentheses refer to the number of patients in each category whose disease presented special problems in chemotherapy.

When the patients were classified according to extent of resection, the incidence of tuberculous complications in each category varied little from the average incidence of 5.4 per cent. When the patients were classified according to regimens of chemotherapy, the incidence varied from 0.77 to 26.3 per cent.

Late results: On January 1, 1959, nearly five years after the first—and six months after the last—resection in the total series of 358 patients, the status of 340 patients (95 per cent) was known (table 6). Three hundred (88.2 per cent) of these patients had inactive disease according to the criteria of the National Tuberculosis Association (5).

Late results are related to the extent of resection in table 6 and to the principal regimens of chemotherapy in table 7. In table 7 the figures in parentheses again show the number of patients in each category who represented problems in chemotherapy.

Of the total number of patients whose status was known on the date of evaluation, there were 17 therapeutic failures (5 per cent), i.e., cases in which the patient died of tuberculous infection or was left with active or active-improved disease. In a few of these cases the failure was relative or a matter of terminology, the disease being classified as active-improved because the exact requirements for inactivity could not be met on the date of evaluation.

In the total of 26 deaths, tuberculous infection was known to be the cause of death in only 3 cases. In 2 cases the cause of death was unknown. Mortality was highest among the patients subjected to pneumonectomy.

TABLE 5

TUBERCULOUS COMPLICATIONS AND CHEMOTHERAPY*

Regimen	Number of Patients	Sputum Positive for Tubercle Bacilli Postoperatively	Increased Disease	Tuberculous Empyema	Patients with Tuberculous Complications†	Complications‡
						per cent
Streptomycin, PAS, isoniazid	258 (28)	2 (2)	2 (2)	0	2 (2)	0.77
Viomycin added at surgery	29 (17)	4 (3)	2 (2)	0	4 (3)	13.8
Viomycin and pyrazinamide	46 (35)	7 (5)	2 (2)	4 (2)	8 (6)	17.4
Cycloserine before surgery	19 (17)	4 (4)	4 (4)	1 (1)	5 (5)	26.3
Patients surviving surgery	352 (97)	17 (14)	10 (10)	5 (3)	19 (16)	5.4

* Figures in parentheses denote resistance to streptomycin and (or) isoniazid and (or) sputum positive for tubercle bacilli at the time of resection.

† Total number who had one or more tuberculous complications.

‡ Per cent of patients surviving surgery who had tuberculous complications.

TABLE 6

PATIENTS SUBJECTED TO RESECTION FEBRUARY, 1954–JULY, 1958
Status January 1, 1959

Procedure	Number of Patients	Status Known*	Inactive†	Active Improved†	Active†	Dead All Causes†	Died of Tuberculosis†
Pneumonectomy	75	72 (96)	56 (77.7)	3 (4.16)	3 (4.16)	9 (12.5)	1
One lung less basal segments	20	20 (100)	19 (95.0)	0	0	1 (5.0)	0
Bilateral resection	17	17 (100)	16 (94.1)	0	0	1 (5.9)	1
Other resections	246	231 (93.9)	209 (90.5)	4 (1.7)	4 (1.7)	15 (6.5)	1
Total	358	340 (95)	300 (88.2)	7 (2.06)	7 (2.06)	26 (7.6)	3 (0.9)

* Figures in parentheses represent per cent of total number of patients.

† Figures in parentheses represent per cent of patients whose status was known.

TABLE 7

CHEMOTHERAPY AND LATE RESULTS OF RESECTION*

Regimen	Number of Patients	Status Known 1/1/59	Inactive	Per Cent Inactive†	Dead, All Causes	Per Cent Dead, All Causes†	Died of Tuberculosis	Active, Active Improved, or Died of Tuberculosis	Per Cent of Active, Active Improved, or Died of Tuberculosis†
Streptomycin, PAS, isoniazid	260 (28)	244 (27)	223 (18)	91.4	16	6.5	0	5 (3)	2.2
Viomycin added	31 (18)	30 (18)	24 (13)	80.0	4	13.3	1 (1)	3 (3)	10.0
Viomycin + pyrazinamide	48 (37)	47 (36)	40 (29)	85.1	4	8.5	0	3 (3)	6.4
Cycloserine	19 (17)	19 (17)	13 (11)	68.4	2	10.5	2 (2)	6 (6)	31.6
Total	358 (100)	340 (98)	300 (71)	88.2	26	7.6	3 (3)	17 (15)	5.0

* Figures in parentheses denote resistance to streptomycin and (or) isoniazid and (or) sputum positive for tubercle bacilli at the time of resection.

† In this table percentages are based on numbers of patients whose status was known.

DISCUSSION

The value of effective chemotherapy in resection for pulmonary tuberculosis was clearly demonstrated in this study. In the group of 258 patients treated with streptomycin, PAS, and isoniazid, among whom problems of chemotherapy were infrequent, only 2 patients (0.77 per cent) had tuberculous complications. Actually, both of these patients had strains of tubercle bacilli resistant to streptomycin and isoniazid and, in retrospect, should have received ancillary medication.

In contrast, among the groups of patients who received viomycin, viomycin and pyrazinamide, and cycloserine, the incidence of complications increased progressively in this order (talbe 5). It must be understood, however, that in this study the incidence of complications with the various regimens was no indication of their comparative efficacy. Rather, the incidence of complications was a reflection of the severity of the patients' chemotherapeutic problems. The cycloserine-treated patients comprised the worst group, 17 (90 per cent) of 19 patients having tubercle bacilli which were highly resistant to the major antituberculous agents and often to the minor ones as well.

As far as complications were concerned, the patient's chemotherapeutic protection appeared to be far more important than the kind of resection to which he was subjected. The percentage of tuberculous complications was no greater among patients who underwent pneumonectomy or other extensive surgery than among those who had more limited resections (table 4).

On the other hand, 16 (16.5 per cent) of 97 patients who had problems of chemotherapy suffered tuberculous complications as compared with 3 (1.2 per cent) of 255 patients who did not have such problems as far as known. (The total of 352 patients who survived surgery included 97 patients in the first group, 255 in the second.) Stated another way, 16 (84 per cent) of the 19 patients who had tuberculous complications were known also to have problems of chemotherapy.

The importance of the patient's chemotherapy is more evident in the early results of pulmonary resection than in the late results, into which so many more factors enter. Nevertheless, in the present series, late as well as early results were best among the patients who had superior chemotherapeutic coverage (table 7). In the group of patients treated with streptomycin, PAS, and isoniazid, among whom problems of chemotherapy were minimal, 91.4 per cent of all patients whose status was known or 97.8 per cent of the patients alive on the date of evaluation had inactive tuberculosis. (Sixteen patients in this group had died, all from causes unrelated to tuberculosis.)

Therapeutic failures increased directly with the severity of the patients' chemotherapeutic problems. Of the total of 17 patients in the series who died of tuberculous infection or who were left with active or active-improved disease, 15 patients (88.2 per cent) had problems of chemotherapy.

The exact value of the ancillary agents and other expedients used in this study cannot be determined from the available data. Such an evaluation was not the purpose of the study which had as its goal, rather, the provision of maximal chemotherapeutic control in every case. With a few exceptions, all patients with strains of *M. tuberculosis* resistant to the major agents and a few patients whose sputum remained positive for tubercle bacilli, despite apparent bacterial susceptibility to and adequate treatment with these agents, were given additional chemotherapy either before, during, or following resection. Thus, there is no group of control cases. Furthermore, in some cases viomycin or viomycin and pyrazinamide were added arbitrarily to the patient's regimen at the time of resection because of prolonged, usually interrupted, previous treatment with streptomycin and isoniazid.

However, the value of viomycin, pyrazinamide, and cycloserine and of other expedients used in this series of cases is apparent in the over-all results. Despite the fact that the total series of 358 patients included 112 patients for whom extensive resection was necessary and 100 patients who had problems of chemotherapy (including 52 patients subjected to extensive resection), tuberculous complications were limited to 5.4 per cent, and only 5.0 per cent of the 340 patients evaluated subsequently could be called therapeutic failures.

In each group of patients who received ancillary agents, the great majority suffered no tuberculous complications, and the great majority were brought to a status of inactivity. Each group included patients who would have succumbed to their disease eventually without definitive treatment in the form of surgical intervention. This is true particularly of the group of patients treated with cycloserine, all of whom were considered to have hopeless disease until treatment with this antimicrobial had brought about sufficient improvement to make pulmonary resection on one or both sides feasible. In nearly all cases in which cycloserine was used, the patient's sputum became negative for tubercle bacilli, and the number of patients in the total series who had infectious sputum at the time of resection was thereby reduced.

The experience of the present writers with the therapeutic efficacy of cycloserine when administered in doses sufficient to produce constant high blood concentrations has been reported elsewhere (6). Cycloserine appears to be the only drug available at the present time which is capable of producing an appreciable therapeutic effect in cases of marked bacterial resistance to streptomycin and isoniazid, and the usefulness of viomycin and pyrazinamide seems to be more or less limited to the protection which they can provide for resectional procedures. In general, to use them otherwise is to waste them because of the problem of bacterial resistance which is always present.

Summary

In a period of nearly five years, 358 patients were subjected to pulmonary resection. A total of 112 patients had extensive resections. A total of 110 patients (including 52 of the patients subjected to extensive resection) had strains of tubercle bacilli resistant to the major antituberculous drugs, streptomycin and isoniazid, and/or infectious sputum at the time of resection.

Chemotherapy was individualized in order to provide maximal chemotherapeutic control. A total of 98 patients received one or more of the so-called minor antituberculous agents, i.e., viomycin, pyrazinamide, and cycloserine, the choice of agents and the timing of their administration depending on the kind and degree of bacterial resistance *in vitro* and the therapeutic goal to be achieved.

Surgical mortality was 1.7 per cent; tuberculous complications, 5.4 per cent. Approximately five years after the first resection in the series, 88.2 per cent of the 340 patients whose status was known had inactive pulmonary tuberculosis. Only 3 of the 26 deaths were caused by tuberculous infection. Including patients who were left with active or active-improved disease, as well as those who died of their infection, a total of 17 patients (5 per cent of all patients whose status was known) were considered to be therapeutic failures.

In respect to tuberculous complications, problems of chemotherapy proved to be far more important than the extent of resection. The incidence of complications was no greater among the patients subjected to pneumonectomy than among those who had less extensive resections. On the other hand, 16.5 per cent of the patients who had problems of chemotherapy suffered tuberculous complications as compared with 1.2

per cent of the patients who did not have such problems as far as was known.

Late as well as early results of pulmonary resection varied inversely with the severity of the patients' chemotherapeutic problems. Yet, even in the groups of patients with the most serious problems, the great majority were brought to a status of inactivity.

The degree of success attained in resections for pulmonary tuberculosis varies directly with the chemotherapeutic control of the patients' disease. By the judicious use of all available chemotherapeutic agents, many patients who have extensive disease and those who have organisms resistant to streptomycin and isoniazid can be treated successfully by resectional surgery.

Sumario

La Quimioterapia por Tuberculosis Pulmonar. Problemas Planteados por la Enfermedad Difundida y los Microbios Fármacorresistentes

En un período de casi cinco años, se sometió a 358 enfermos a la resección pulmonar. En un total de 112 enfermos las resecciones fueron extensas. Un total de 110 enfermos (comprendiendo a 55 de los enfermos cuyas resecciones fueron extensas) tenía cepas de bacilos tuberculosos resistentes a los mayores medicamentos antituberculosos, estreptomicina e isoniacida, y/o tenían esputo infeccioso al hacerse la resección.

Se individualizó la quimioterapia a fin de obtener el dominio quimioterapéutico máximo. Un total de 98 enfermos recibió uno o más de los llamados agentes antituberculosos menores, es decir, viomicina, piracinamida y cicloserina, basándose la selección de medicamentos y el horario de su administración en la clase e intensidad de la resistencia bacteriana *in vitro* y en la meta terapéutica en mira.

La mortalidad quirúrgica fué de 1.7 por ciento; las complicaciones tuberculosas representaron 5.4 por ciento. Aproximadamente a los cinco años de la primera resección de la serie, 88.2 por ciento de los 340 enfermos cuyo estado se conocía tenían tuberculosis pulmonar inactiva. Solamente 3 de las 26 muertes habían sido ocasionadas por infección tuberculosa. Incluyendo a los enfermos que habían quedado con enfermedad activa o activa-mejorada, así como a todos los que fallecieron de su infección, se consideró que un total de 17 enfermos (5 por ciento de todos aquéllos cuyo estado se conocía) representaba fracasos terapéuticos.

Con respecto a complicaciones tuberculosas, los problemas de la quimioterapia resultaron ser mucho más importantes que el tamaño de la resección. La incidencia de complicaciones no fué mayor entre los sujetos sometidos a la neumonectomía que en aquéllos en quienes se hicieron resecciones menos extensas. Por otro lado, 16.5 por ciento de los enfermos que tuvieron problemas quimioterapéuticos experimentaron complicaciones tuberculosas, comparado con 1.2 por ciento en los enfermos que, en lo que se sepa, no tuvieron tales problemas.

Los resultados tanto tardíos como tempranos de la resección pulmonar variaron en razón inversa a la gravedad de los problemas quimioterapéuticos de los enfermos. No obstante, aun en los grupos de enfermos que tenían los problemas más graves, se llevó a la mayoría a un estado de inactividad.

La magnitud del éxito alcanzado en las resecciones por tuberculosis pulmonar varía en razón directa al dominio quimioterapéutico de la enfermedad del sujeto. Con el empleo juicioso de todos los agentes quimioterapéuticos disponibles, cabe tratar con éxito por medio de la resección quirúrgica a muchos enfermos que tienen enfermedad difundida y a los que tienen microbios resistentes a ls estreptomicina y la isoniacida.

Resume

Chimiothérapie dans la résection pour tuberculose pulmonaire. Problème d'une forme extensive avec germes résistant aux médicaments

Dans une période de près de cinq ans, 358 malades furent soumis à une résection pulmonaire. Un total de 112 malades eurent des résections étendues. Un total de 110 malades (y compris 52 des malades soumis à une résection étendue) avaient des souches de bacilles tuberculeux résistant à la plupart des agents anti-tuberculeux, streptomycine et isoniazide, et/ou avaient une expectoration positive au moment de la résection.

La chimiothérapie fut individualisée pour procurer un contrôle maximum de chimiothérapie. Un total de 98 malades reçut un ou plus des agents antituberculeux dits "mineurs", c'est à dire, viomycine, pyrazinamide et cyclosérine, le choix des agents et le rythme de leur administration dépendant du genre et du degré de la résistance bactérienne *in vitro* et du but thérapeutique à atteindre.

La mortalité chirurgicale fut de 1,7%; les complications tuberculeuses de 5,4%. Après à peu près cinq ans, la première résection dans ces séries, 88,2% des 340 malades dont l'état connu, avaient une tuberculose pulmonaire inactive. Seulement 3 des 26 morts furent causées par l'infection tuberculeuse. En comprenant les mala-

des qui furent laissés avec une forme active, ou active améliorée, ainsi que ceux qui moururent de leur infection, un total de 17 malades (5% de tous les malades dont l'état était connu) fut considéré comme un échec thérapeutique.

Au point de vue des complications tuberculeuses, les problèmes de chimiothérapie se sont avérés être beaucoup plus importants que l'étendue de la résection. La fréquence des complications ne fut pas plus grande parmi les malades soumis à une pneumectomie que parmi ceux qui eurent des résections moins étendues. D'autre part, 16,5% des malades qui présentèrent des problèmes thérapeutiques, eurent des complications tuberculeuses, en comparaison de 1,2% chez les malades qui n'eurent pas ces problèmes-là, autant qu'on l'ait su.

Les résultats précoces connus, comme les résultats tardifs de résection pulmonaire varient inversement avec la gravité des problèmes thérapeutiques des malades. Cependant, même dans le groupe de malades ayant les problèmes les plus sérieux, la grande majorité fut amenée à un état d'inactivité.

Le degré de succés obtenu dans les résections pour tuberculose pulmonaire varie directement avec le contrôle thérapeutique de la maladie de ces malades. Par l'emploi judiçieux de tous les agents chimiothérapiques dont on peut disposer, beaucoup de malades qui ont une forme étendue, ou qui ont des germes résistants à la streptomycine et à l'isoniazide, peuvent être traités avec succés par une résection chirurgicale.

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SERUM ELECTROPHORESIS IN SARCOIDOSIS, TUBERCULOSIS, AND DISEASE DUE TO UNCLASSIFIED MYCOBACTERIA[1, 2]

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INTRODUCTION

A characteristic electrophoretic pattern of serum proteins has been described in patients with clinical tuberculosis (1) and in those with active sarcoidosis (2). The major difference between the two is the predominating increase in the alpha-2 globulin in tuberculosis and the increase in gamma globulin in sarcoidosis. In the latter disease, there is frequently an increase in other globulin fractions, producing a steplike pattern.

During the course of an investigation of alterations of serum protein in a number of patients with sarcoidosis, it was possible to study one patient with this disease who also developed pulmonary tuberculosis and another who developed pulmonary disease caused by an unclassified Mycobacterium (nonphotochromogenic). The electrophoretic pattern of the first patient changed from a steplike pattern to one with an elevated alpha-2 globulin, but that of the second did not change (figure 1). This finding led the writers to investigate and compare the electrophoretic patterns of serum from a number of patients with sarcoidosis, tuberculosis, and unclassified mycobacterial infection. The quantitative values of the various fractions were also compared.

MATERIALS AND METHODS

Specimens of serum were obtained from: 44 cases[3] of sarcoidosis, proved by biopsy; 31 cases[4] of tuberculosis; and 74 cases[4] of unclassified mycobacterial infection, proved by culture of sputum or tissue; 14 cases[4] of mixed tuberculosis and unclassified mycobacterial disease; and 32 "normal" donors.[5] Specimens sent by mail contained a 1:10,000 dilution of merthiolate as preservative, and all sera were stored in the frozen state and thawed just prior to testing. Electrophoretic separation was accomplished by use of the Spinco Durrum type of paper electrophoresis apparatus. The paper strips were stained with methyl alcohol solution of bromphenol blue and evaluated by scanning in the Model RB Analytrol. Total protein concentration was determined by the biuret method of Weichselbaum (3).

The significance of the deviations from the comparison group was computed by the "t" test (4). All sera were examined and results were computed without knowledge of the diagnosis. The patterns were classified into three groups: "steplike," "tuberculosis," and "normal." The label "steplike" was attached if (*1*) there was a definite steplike configuration of the fraction peaks, and (*2*) the pattern terminated in an elevated gamma globulin. The term "tuberculosis" was assigned to patterns showing an elevated alpha-2 globulin peak, standing above either or both the beta or gamma globulin peaks. The others were termed "normal" if the pattern was not characteristic of either group above, whether or not some values might be slightly abnormal.

RESULTS

Quantitative values: The elevation in the total protein and gamma globulin and the decrease in albumin and A/G ratio (table 1) found in the cases of sarcoidosis are in agreement with previous studies (2, 5, 6). The tuberculosis group had the lowest mean albumin and the highest alpha-2 and gamma globulin values. Baldwin and Iland (1), Jencks and associates (6), and others have noted these changes, but not this degree of hypergammaglobulinemia. Elimination of inactive cases did not significantly change the mean globulin values (table 1).

The values in unclassified mycobacterial infection showed lower alpha-2 and gamma globulin levels and a higher albumin than those in the group with tuberculosis. The value of all fractions was similar to that in sarcoidosis, these two groups being the only ones with significant

[1] From the Veterans Administration Research Laboratory and Clinic, and the Jacksonville Hospitals Educational Program, Inc., Jacksonville, Florida.

[2] Presented before the Medical Session at the Annual Meeting of the Southern Trudeau Society, October 1, 1959.

[3] From patients in the Veterans Administration Sarcoidosis Research Clinic, Jacksonville, Florida.

[4] From the Florida State Tuberculosis Hospitals.

[5] From the Jacksonville Blood Bank, Jacksonville, Florida.

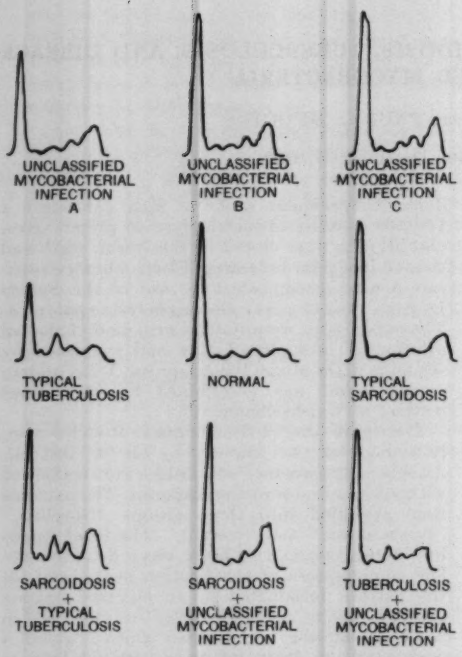

FIGURE 1. The electrophoretic patterns, carefully reproduced, from cases included in the study.

decreases in A/G ratio. The patients who had both tuberculosis and unclassified mycobacterial infection did not have distinctive changes as a group. In fact, none of the mean values showed a significant deviation from normal. However, some individual cases in this varied group had abnormalities ranging from increased alpha-2, beta, or gamma globulins to various combinations of these. Because there has been no previously published report in the English literature on serum electrophoresis in cases of unclassified mycobacterial infection, the values for this group are listed in table 2 in per cent of total protein, grams per 100 ml., and range. The mean quantitative values on all groups, including the normal subjects, showed a steplike increase from the alpha-1 to the gamma globulin; yet the electrophoretic patterns showed a steplike increase in peaks only for the cases of sarcoidosis and unclassified mycobacterial infection.

Visual electrophoretic patterns: The results in the various groups are seen in table 3. The incidence of steplike patterns in unclassified mycobacterial infection was twice as high as in tuberculosis and almost three times as high as the incidence of tuberculosis patterns in the same group (unclassified mycobacterial infection). There were more normal patterns in the unclassified mycobacterial group than in the tuberculosis

TABLE 1

MEAN VALUES OF SERUM PROTEIN FRACTIONS AND VARIATIONS DUE TO DISEASE ACTIVITY IN SARCOIDOSIS, TUBERCULOSIS, AND UNCLASSIFIED MYCOBACTERIAL INFECTION

	Number of Cases	Total Protein	A/G Ratio	Albumin	Globulins			
					A-1	A-2	Beta	Gamma
		gm./100 ml.		*per cent*	*per cent*			
Normal subjects	32	7.0	2.1	67.0	4.3	6.5	9.1	12.2
Sarcoidosis								
Active	30	8.2*	1.6*	58.7*	4.3	6.9	9.7	20.8*
Inactive	14	7.8*	2.2*	68.0*	3.8	5.8	7.5	13.7*
Mean	44	8.1*	1.7*	61.7*	4.2	6.2	9.0	18.6*
Tuberculosis								
Active	27	7.2*	1.1	49.3	6.0	10.1	10.8	23.6
Inactive	5	6.9*	1.0	47.0	7.6	9.1	11.2	24.9
Mean	32	7.2	1.1	49.1*	6.2	9.8*	10.7	23.7*
Unclassified mycobacterial infection								
Active	64	7.0	1.5	56.9	6.2	8.6	8.9	19.2
Inactive	10	6.8	1.7	59.8	6.3	7.5	9.2	17.2
Mean	74	7.0	1.5*	57.4	6.2	8.4	9.0	18.9*
Both tuberculosis and unclassified mycobacterial infection	14	6.4	1.3	55.1	6.8	10.0	9.2	18.9

* Significant at $P = <0.01$.

TABLE 2

VALUES OF SERUM PROTEIN FRACTIONS IN 74 CASES OF UNCLASSIFIED MYCOBACTERIAL INFECTION

	Total Protein	A/G Ratio	Albumin	Globulins			
				Alpha-1	Alpha-2	Beta	Gamma
Mean:							
Grams per 100 ml.*	7.0	1.5	4.0	0.4	0.6	0.6	1.4
Per cent of total protein	—	—	57.4	6.2	8.4	9.0	18.9
Range:							
Grams per 100 ml.*	5.5–9.8	0.5–3.6	2.2–6.3	0.2–0.9	0.3–1.4	0.4–1.0	0.4–3.6

* Except for A/G ratio.

TABLE 3

OCCURRENCE OF THE VARIOUS ELECTROPHORETIC PATTERNS

Diagnosis	Total Cases	Type of Pattern (Per Cent of Total Cases)		
		Steplike	Tuberculosis	Normal
Normal subjects	32	3	0	97
Sarcoidosis	44	70	7	23
Tuberculosis	32	28	66	6
Unclassified mycobacterial infection	74	55	22	23
Tuberculosis and unclassified mycobacterial infection	14	36	50	14

TABLE 4

ELECTROPHORETIC PATTERN IN VARIOUS TYPES OF UNCLASSIFIED MYCOBACTERIAL INFECTION

Type of Organism	Number	Type of Pattern (Number)		
		Steplike	Tuberculosis	Normal
Nonphotochrome	58	31	13	14
Scotochrome	12	7	2	3
Photochrome	4	2	2	0

group. The patients infected with both *M. tuberculosis* and unclassified mycobacteria showed an almost equal incidence for the steplike and tuberculosis patterns, but it is difficult to draw any significant conclusions from such a varied group. Of definite significance, however, is the finding of three times as many tuberculosis patterns in the tuberculosis group as in the group with unclassified mycobacterial infection. There were several cases of tuberculosis with steplike patterns, and undoubtedly similar patterns will be seen in many other diseases on occasion.

Most of the unclassified mycobacteria were nonphotochromogenic (Runyon's *Group III*). However, there were a few cases with scotochromes (*Group II*) and photochromes (*Group I*). The number of the various types of patterns seen in the different types of unclassified mycobacterial infections is shown in table 4. The steplike pattern was prevalent in all three groups.

Because of the similarity of patterns in sarcoidosis and unclassified mycobacterial infections, an attempt was made to discover any common factor(s) among such cases, clinical or otherwise. Certainly, the age and sex distributions were dissimilar. Sarcoidosis is a disease that predominates in young Negroes, with equal sex distribution, while infection with unclassified mycobacteria occurs chiefly in elderly white males (7). The distribution of age, sex, and race among the patients may be seen in table 5.

DISCUSSION

The comparison of quantitative values of the serum protein fractions in cases of sarcoidosis and tuberculosis demonstrated expected changes from normal. The electrophoretic study of serum proteins has not previously been reported in infection with unclassified mycobacteria. In 74 cases, the changes were more like those in sarcoidosis than tuberculosis, with a decrease in serum albumin and an increase in gamma globulin, without an increase in alpha-2 globulin. However, looking at the quantitative values will frequently not give a lead as to the appearance of the electrophoretic pattern. In some diseases the quantitative values are so distinctly abnormal as to suggest a diagnosis, and this is true in multiple

TABLE 5

DISTRIBUTION BY AGE, SEX, AND RACE

Disease	Number	Mean Age at Onset	Males	Negro	Active Disease
			per cent	*per cent*	*per cent*
Sarcoidosis	44	29.2	52.4	70.5	68
Unclassified mycobacterial infection	74	50.0	62.0	30.0	88
Tuberculosis	32	46.0	78.0	53.0	84

myeloma and the nephrotic syndrome. But often the values in two cases may be similar, yet the patterns have a very different appearance. This is caused by the difference in "spread" of the protein fractions, so that a high quantitative value may be spread in such a way that the peak is lower than the adjacent fraction with a lower quantitative value. On analysis of the patterns, even further evidence of a similarity between sarcoidosis and unclassified mycobacterial infection was found. It is not possible to give an adequate explanation for this finding.

The frequent similarity of the patterns in unclassified mycobacterial infection and sarcoidosis makes one inquisitive about the similarities of other features of those diseases. There is considerable difference in the roentgenographic changes. Bilateral hilar lymphadenopathy is common in sarcoidosis, as is generalized parenchymal involvement in the form of infiltrates and fibrosis. The infiltrates are frequently transient and even migratory. The chest film in unclassified mycobacterial infection is more like that of typical tuberculosis, with localized infiltrates particularly in the upper lobes, and frequent cavitation. There have been no reports of bilateral hilar adenopathy in this disease. There is a tendency to mild chronic disease in both sarcoidosis and unclassified mycobacterial infection. Organ involvement is also very different, with lung, skin, lymph node, bone, liver, and spleen involvement in sarcoidosis, while unclassified mycobacterial infection is usually restricted to the lungs (7). On the other hand, a reason is sought for the difference in the electrophoretic pattern in tuberculosis and unclassified mycobacterial infection when the roentgenograms and clinical features are so similar. There is no difference in the incidence of severe cases or the duration of disease in the two groups that would explain this difference in patterns.

Although not adapted to routine use as a laboratory test, it appears that evaluation of the electrophoretic pattern in some problem cases of pulmonary disease would have diagnostic significance. This is especially true when viewed in the light of other clinical data, such as skin tests, roentgenograms, and bacteriologic procedures.

SUMMARY

The results of serum protein electrophoresis done on patients with sarcoidosis, tuberculosis, and unclassified mycobacterial infection have been presented. Emphasis is placed on the diagnostic value of the pattern as opposed to the quantitative values, with the understanding that the results obtained are important only when evaluated with the total clinical picture. In this study, an evaluation of electrophoretic patterns has brought out the similarity in unclassified mycobacterial infection and sarcoidosis and the dissimilarity of unclassified mycobacterial infection and tuberculosis.

SUMARIO

La Electroforesis Sérica en la Sarcoidosis, la Tuberculosis y la Debida a Micobacterias Inclasificadas

Preséntanse los resultados de la electroforesis de la seroalbúmina en enfermos de sarcoidosis, tuberculosis y de infección micobacteriana inclasificada. Recálcase el valor diacrítico de este patrón en contraposición a los valores cuantitativos, sobreentiéndose que los resultados obtenidos no son importante más que cuando se justiprecian junto con el cuadro clínico total. En este estudio, una justipreciación de los patrones electroforéticos ha puesto de manifiesto la semejanza entre la infección micobacteriana inclasificada y la sarcoidosis y la desemejanza entre la infección micobacteriana inclasificada y la tuberculosis.

RESUME

Electrophorèse du sérum dans la sarcoïdose, la tuberculose, et une infection due à des mycobactéries non classées

L'auteur présente les résultats de l'électrophorèse des protéines sériques effectuées chez des

malades ayant une sarcoïdose, une tuberculose et une infection à mycobactéries non classées. L'auteur insiste sur la valeur diagnostique du type d'électrophorèse en le comparant aux valeurs quantitatives, en comprenant que les résultats obtenus sont importants seulement quand ils sont évalués avec tout le contexte clinique. Dans cette étude, une évaluation des types d'électrophorèse a mis en évidence la similarité entre la sarcoïdose et les infections à mycobactéries non classées, et la différence entre la tuberculose et les infections à mycobactéries non classées.

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STUDIES ON NOCARDIA AND OTHER ACTINOMYCETALES[1, 2]

II. Antigenic Relationships Shown by Slide Agglutination Tests

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Introduction

Immunologic investigations on the aerobic Actinomycetales have been carried out for many years, but most of the older comparative studies are of limited value since they involved only a few species or strains, the identity of which cannot be correlated with currently recognized species. Some of the earlier researches were confined largely to analysis of allergic manifestations provoked in experimental animals, but such *in vitro* methods as agglutination, precipitation, and complement-fixation have also been employed. From examination of the data obtained in previous serologic studies, utilizing recognizable strains of Nocardia, Streptomyces, and Mycobacterium (1–9), the general conclusion may be drawn that, while some acid-fast Nocardia species share common antigenic factors which can also be found in certain species of Mycobacterium, both acid-fast nocardiae and mycobacteria seem to have significantly less, if any, antigenic relationship with species of Streptomyces or with nonacid-fast, nonfragmenting streptomyces-like strains presently classified as Nocardia.

In a former publication from this laboratory (10) were described the results of comparative cultural studies which were carried out with 51 strains received as Nocardia, as well as with smaller numbers of strains designated as Mycobacterium and Streptomyces species, respectively. On the basis of the data obtained, the strains of Nocardia were separated into the "streptomyces-like Nocardia" and "true Nocardia," the latter being further divided into four major groups designated as follows: *Nocardia asteroides* group; *N. brasiliensis* group; *N. corallina* group; and *N. opaca* group. The present study was undertaken in order to investigate further (using the method of slide agglutination with cell suspensions and antiserum prepared for many of the strains collected) antigenic relationships among members within the genus Nocardia and related organisms, particularly the Mycobacterium species. It was planned also to determine to what extent the relationships thus uncovered would correlate with the groupings previously set up on the basis of cultural similarities.

Materials and Methods

The following strains, previously described as to source and designation (10), were used here: *N. asteroides* (18 strains), *N. blackwellii*, *N. brasiliensis* (6 strains), *N. caprae*, *N. corallina*, *N. cuniculi*, *N. erythropolis*, *N. gardneri*, *N. globerula*, *N. intracellularis*, *N. leishmanii*, *N. madurae* (3 strains), *N. minima*, *N. opaca*, *N. paraffinae*, *N. pelletieri* (2 strains), *N. polychromogenes*, *N. rangoonensis*, *N. rubra* (2 strains), *Mycobacterium butyricum*, *M. fortuitum*, *M. smegmatis* (2 strains), *M. phlei* (2 strains), *Streptomyces griseus*, and *S. ruber*. Additional strains studied, not previously described, were: *Mycobacterium fortuitum* strains NCTC 8697 and R389 (a),[3] *M. phlei* R5 (a), *M. smegmatis* strains R20 and R3 (a), *M. tuberculosis* (avian) strains 210 (c) and 4017-2(d), *M. tuberculosis* (bovine) strains Ravenel and 4288-4(b), *M. tuberculosis* (human) strains H37Rv and Erdman (b), Jy 135, Au 234, and 87280 (e).

All Nocardia and Streptomyces strains were cultivated in a uniform semisynthetic modification of the Long-Seibert fluid medium with the following composition: Casamino acids (Difco), 10.0 gm.; L-asparagine, 0.2 gm.; potassium acid phosphate, 1.5 gm.; magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.25 gm.; ferric ammonium citrate, 0.01 gm.; D-mannose, 5.0 gm.; glycerol, 40.0 ml.; distilled water, 1 liter. For each strain, four to six flasks, each containing 100 ml. of sterile medium, were inoculated by seeding the surface with an emulsified inoculum from cultures on heart-infusion glycerol agar. Flasks were incubated at 25 to 30°C. for four weeks, following which the growth

[1] From the Department of Microbiology, Tulane University School of Medicine, New Orleans, Louisiana.

[2] This work was supported by a contract between the Office of Naval Research, Department of the Navy, and Tulane University.

[3] The sources indicated by the letters in parentheses were: (a) Dr. R. Gordon, Rutgers University; (b) Dr. W. Steenken, Jr., Trudeau Foundation; (c) Dr. F. R. Beaudette, Rutgers University; (d) Dr. A. G. Karlson, Mayo Clinic; and (e) Ochsner Foundation Hospital, New Orleans.

from each strain was pooled. Phenol was added to 1 per cent final concentration, and the pools were stored at 25°C. for at least three days before use.

For each strain, the pooled phenolized growth was poured through sterile filter paper and the microbial mass retained was used to prepare the following antigen suspensions which were stored in the refrigerator until needed.

Oily suspension: One gram (wet weight) of mycelium was ground in a mortar with 15 to 20 ml. of sterile mineral oil (added gradually) until a nearly homogenous emulsion was obtained. Strains difficult to emulsify in this manner, e.g., some strains of *N. brasiliensis* and streptomyces-like Nocardia, were first ground in the mortar with a small amount of saline, then with oil added as previously described.

Saline suspension: The remaining filtered microbial mass was placed in a separate mortar and ground vigorously for 15 to 20 minutes with one to two times its volume of washed sterile Celite[4] abrasive to make a relatively dry stiff paste. Then at intervals during grinding, small aliquots of "antigen diluent"[5] were added until a creamy suspension was obtained. This was diluted to approximately 100 ml. with the same antigen diluent, further homogenized in a Waring Blendor for ten minutes, made up to a final volume of 150 ml. with additional diluent, and then centrifuged at 600–700 r.p.m. for ten minutes. The supernate was divided into two equal aliquots, one ("immunizing antigen") being eventually used without further modification in the immunization procedures. The second aliquot, designated "agglutinating antigen," was adjusted with the aid of a Lumetron photometer to final optical density equivalent to that of a suspension of *E. coli* containing 2×10^9 cells per ml. It may be noted here that all such attempts to prepare stable suspensions of *N. caviae* were unsuccessful; the ground material consistently exhibited rapid spontaneous agglutination upon addition of the diluent.

Prior to immunization, all animals were bled and the serum was stored at 4 to 5°C. for later use as a control specimen. Both control and immune sera were preserved with phenol added to 0.25 per cent final concentration. The rabbits were ordinarily immunized according to the following schedule. Each was given 4 ml. of oily suspension subcutaneously (divided equally in four separate sites) and at the same time 3 ml. of saline antigen suspension intravenously. After four additional such intravenous injections, at forty-eight-hour intervals, three injections similar in dose and spacing were administered following a twelve-day rest period. Animals were bled one to two weeks thereafter, providing the serum agglutination titer had reached a level of 1:80 (initial serum dilution) or higher for the homologous antigen; otherwise a booster series of three more injections was given on alternate days, beginning three weeks after completion of the above basic schedule. Some animals received a second similar booster series, after which they were bled regardless of serum titer.

A pair of rabbits was immunized with certain strains in order to have serum from two different animals for comparison; however, for most strains only one rabbit was used unless the first failed to produce antiserum of sufficient titer. Bleedings of equivalent titer from the same animal were pooled; aliquots were stored at −40°C. and 4°C., respectively, the latter being used to perform the agglutination tests. Since *N. caviae* agglutinated spontaneously in saline suspension, the immunization procedure for this strain consisted of subcutaneous injections of 4 ml. of oily suspension, followed by 3 ml. of saline suspension given intraperitoneally (to avoid possible death from emboli) every fifth day for a total of seven injections.

The antigen suspensions and antiserum for Mycobacterium strains were prepared, in connection with another study, by methods similar to those described above for Nocardia and Streptomyces except that Alundum® was used for grinding instead of Celite. These serologic reagents were kindly furnished by Dr. Leah S. Shaffer (now deceased).

The agglutination tests were performed on glass plates[6] measuring 138 × 138 mm., with 36 circular flat-bottomed wells, 1.3 mm. deep and 15 mm. in diameter. The undiluted standardized antigen suspensions were tested against all immune serum available, the latter having been made up with buffered saline in doubling dilutions ranging from 1:5 to 1:1,280. Pre-immunization serum was employed in a 1:5 dilution. Equal volumes (0.06 ml.) of antigen and serum dilution were placed together in the wells, and the plates holding the serum-antigen mixtures were rotated at room temperature on a Clay-Adams variable-speed horizontal rotator for ten minutes at 50 to 70 r.p.m. They were then inspected for grossly visible agglutination by holding the plate against a black background with oblique illumination from a fluorescent lamp. Many mixtures showed clumps after two to three minutes of rotation; others required more time, but rotation beyond ten minutes did not increase the titers and all positive reactions became definitely established within this interval. Clumping that required a hand lens to see, or that was otherwise questionable, was considered negative. Whenever an immune serum showed a prozone effect with clumping only in the higher dilutions, the preimmunization serum was tested in a parallel series of dilutions. Each immune serum was titrated against all available antigen suspensions on a single day and was also

[4] A diatomaceous earth filter-aid prepared and marketed by the Johns-Manville Company.

[5] Fifteen-hundredths M NaCl solution buffered at pH 7.2 to 7.4 with 0.025 M potassium phosphate and containing 0.3 per cent phenol plus 0.1 per cent sodium taurocholate (Difco).

[6] J. M. Freed Co., Perkasie, Pennsylvania, Catalogue No. 2036 D.

TABLE 1

SLIDE AGGLUTINATION TITERS IN TESTS WITH ANTISERA VS. N. ASTEROIDES GROUP

Antigens from Strains of:	Antisera vs. Nocardia															
	Asteroides Strains										*N. blackwellii*	*N. caprae*	*N. cuniculi*	*N. minima*	*N. paraffinae*	*N. polychromogenes*
	H.4139	ATCC 3308	CDC A343	14A1	47295	2001	356	ATCC 9969	359	NRRL B970						
Nocardia																
asteroides strains:*																
Henrici 4139	*80*	80	20	40	20	40	20	40	20	40	40	40	40	40	20	40
ATCC 3308	40	*320*	20	160	80	80	80	80	40	40	80	320	160	80	80	160
CDC A343	—	40	*80*	80	40	20	20	20	10	40	40	80	40	80	40	40
14A1	20	320	40	*160*	160	320	80	80	20	80	80	160	160	80	80	80
47295	10	160	20	80	*160*	80	40	40	10	40	40	80	80	40	40	40
2001	5	160	10	40	160	*80*	40	40	20	40	80	80	80	40	40	40
356	20	160	10	40	80	80	*80*	40	20	20	80	80	80	80	20	40
ATCC 9969	20	320	20	160	80	80	40	*80*	20	20	80	160	80	80	40	40
359	20	160	20	80	40	80	80	40	*160*	40	160	160	80	80	40	80
NRRL B970	—	80	20	40	20	40	40	20	10	*80*	10	40	20	40	80	20
blackwellii	10	160	10	80	40	80	40	80	20	20	*80*	80	80	40	20	20
caprae	—	20	—	10	5	10	—	10	—	—	40	*40*	5	—	10	10
cuniculi	—	320	—	160	160	80	40	80	20	40	80	80	*160*	40	80	80
minima	—	40	—	20	5	10	—	10	—	—	10	80	20	*40*	20	—
paraffinae	—	40	—	20	5	40	10	40	—	—	—	20	20	40	*40*	10
polychromogenes	—	40	—	10	10	10	—	20	5	—	—	40	5	—	10	*40*
brasiliensis strains:																
Ochoa 409	—	5	—	—	—	—	—	—	—	—	—	—	—	—	10	5
2178	—	40	—	—	5	—	—	10	—	—	—	40	—	—	20	20
Ochoa 414	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	10
47296	—	40	—	10	10	40	10	40	10	10	5	40	10	10	10	20
992	—	10	—	—	—	—	—	10	—	—	10	—	—	—	—	—
Ochoa 413	—	5	10	—	—	—	—	—	—	—	—	—	5	—	—	—
corallina	—	320	20	80	40	80	40	80	10	20	40	160	80	40	20	20
erythropolis	10	320	40	320	40	80	40	80	10	40	40	80	80	40	40	20
globerula	—	160	10	160	80	80	40	80	10	20	40	80	80	40	20	10
opaca	—	80	—	40	80	40	20	80	20	—	—	40	80	20	40	40
rubra—NRRL B635	—	40	—	—	—	—	—	40	—	—	—	40	—	—	40	40
rubra-379	—	160	—	40	20	80	20	80	40	80	80	80	80	40	80	20
intracellularis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Streptomyces-like Nocardia†	—	5	—	—	—	—	—	—	—	—	—	—	10	—	10	—
Streptomyces																
griseus	—	5	—	—	—	—	—	—	—	—	—	—	—	—	10	10
ruber	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mycobacterium																
butyricum	—	—	—	—	—	—	—	5	—	5	—	—	—	—	10	5
fortuitum—ATCC 6841	—	160	20	40	20	20	10	20	10	40	—	20	20	10	40	10
fortuitum—NCTC 8697	—	80	10	—	20	40	10	20	10	80	20	20	20	20	40	10
fortuitum—R389	—	160	20	40	10	40	10	40	10	40	—	20	10	—	20	10
phlei—R5	—	—	—	—	10	—	—	10	10	80	—	—	10	—	80	10
phlei—W23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
smegmatis‡—270	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—
smegmatis‡—NCTC 8150	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Also tested: strains Wojik 23, Henrici A10A, ATCC 9504, ATCC 9970, CDC-A402, CDC-47, CDC-144, CDC-A267. Their pattern of cross reactivity was almost identical to that exhibited by the strains of *N. asteroides* listed in the table. The results have been omitted for the sake of brevity.

† This group includes all strains listed as "Streptomyces-like Nocardia" in table 3. The figures in tables 1 and 2 represent a composite of the reactivity of the nine strains in the group.

‡ Also tested: strains R3 and R20. Cross reactivity was similar to that exhibited by strains 270 and NCTC 8150.

Italicized figures indicate the homologous titers.

TABLE 1—*Continued*

Antigen from Strains of:	Antisera vs. Nocardia															
	Asteroides Strains										*N. blackwellii*	*N. caprae*	*N. cuniculi*	*N. minima*	*N. paraffinae*	*N. polychromogenes*
	H.4139	ATCC 3308	CDC A343	14A1	47295	2001	356	ATCC 9969	359	NRRL B970						
tuberculosis (avian)—210	—	80	—	—	—	—	—	40	—	—	—	—	—	—	10	—
tuberculosis (avian)—4017-2	—	80	—	10	—	—	—	40	5	20	—	—	5	—	20	5
tuberculosis (bovine)—Ravenel	—	80	—	20	—	10	—	40	10	20	—	20	10	—	20	10
tuberculosis (bovine)—4228-4	—	320	—	40	—	20	—	40	10	20	—	—	—	—	20	20
tuberculosis (human)§—H37Rv	—	40	—	10	—	10	—	10	—	—	—	10	—	—	5	—
tuberculosis (human)§—Erdman	—	80	—	5	—	20	—	10	—	—	—	10	—	—	10	5

§ Also tested: strains Jy135, Au 234, and 87280. Cross reactivity was similar to that exhibited by strains H37Rv and Erdman.

tested against a suspension of Celite abrasive in antigen diluent as an additional control; the tests were repeated at least once, sometimes twice, to check the reproducibility of results.

Results

Serologic response of rabbits to immunization schedule: It may be stated at the outset that the control pre-immunization bleedings of all of the animals used were found to show no gross agglutination at the lowest dilution (1:5) tested.

For most strains of *N. asteroides*, a homologous serum titer of at least 1:80 was attained by the end of the basic immunization period. Frequently, bleedings taken seven days after the last injection of the primary series (only fifteen days from the start of immunization) showed titers of 1:40 or greater, indicating a relatively rapid antibody response. Once a peak value was reached following a given series of injections, successive bleedings collected over the course of the next fortnight usually showed no further increase or even a minor decline in the level. The animal receiving strain CDC A 267 required a single booster series to attain 1:80 titer, while strains Henrici A 101 A and CDC A 144 failed to evoke a titer greater than 1:40, despite repeated series of booster injections and the use of additional animals.

All but one strain of *N. brasiliensis* produced satisfactory titers as a result of the basic immunization. Strain 47296 did not evoke a significant antibody in the first rabbit despite repeated booster doses, but a titer of 1:40 was obtained in a second animal.

Antiserum considered adequate for the purpose was obtained for the other Nocardia and the mycobacteria used, either by the end of the basic immunization period or following a single booster series.

With antigens prepared from the 2 Streptomyces species or the strains of streptomyces-like Nocardia, it was usually necessary to employ two booster series in order to obtain antiserum with titers of 1:40 or greater.

Tests with antisera vs. members of N. asteroides group: In table 1 are presented the titers observed when antigens of Nocardia, Mycobacterium, and Streptomyces species were tested with immune sera vs. strains previously classified on the basis of cultural data as belonging to the *N. asteroides* group. It will be noted that, while marked reciprocal cross agglutination was common among *N. asteroides* strains, the *N. brasiliensis* strains were seldom agglutinated to a comparable titer. The suspensions of *N. blackwellii* and *N. cuniculi* reacted with the various antisera in a manner paralleling that of *N. asteroides* strains. However, *N. caprae*, *N. minima*, *N. paraffinae*, and *N. polychromogenes* suspensions were less frequently clumped at higher dilutions by heterologous

TABLE 2

SLIDE AGGLUTINATION TITERS IN TESTS WITH ANTISERA VS. CERTAIN NOCARDIA AND MYCOBACTERIA

Antigens from Strains of:[a]	Antisera vs. Nocardia: *N. brasiliensis* Ochoa 409	*N. brasiliensis* 2178	*N. brasiliensis* Ochoa 414	*N. brasiliensis* 47296	*N. brasiliensis* 992	*N. brasiliensis* Ochoa 413	*N. corallina*	*N. globerula*	*N. rubra* NRRL B685	*N. rubra* 379	*N. intracellularis*	Mycobacterium *M. fortuitum* ATCC 6841	*M. phlei* 125	*M. smegmatis* NCTC 8150	*M. tuberculosis* avian—4017.2	*M. tuberculosis* bovine—4228.4	*M. tuberculosis* human—H37Rv
Nocardia asteroides strains:[b]																	
Henrici 4139	40	40	10	5	40	20	—	10	80	20	—	—	—	—	—	—	—
ATCC 3308	5	160	160	40	160	40	40	80	80	40	—	40	40	20	—	20	20
CDC A343	—	80	40	20	80	20	5	10	80	40	—	—	10	—	—	—	—
14A1	—	80	160	10	160	40	80	80	80	20	—	—	10	—	—	—	5
47295	—	80	40	40	80	20	10	20	40	20	—	—	5	—	—	—	—
2001	—	80	80	80	160	20	5	40	40	20	—	—	20	—	—	—	5
356	—	80	40	80	80	10	—	20	40	10	—	—	—	—	—	—	—
ATCC 9969	40	80	40	40	160	20	40	40	80	20	—	—	10	—	—	—	—
359	—	80	40	40	80	20	5	20	80	10	10	—	—	—	—	—	40
NRRL B970	20	20	10	10	40	20	—	10	20	20	5	—	40	—	40	20	20
blackwellii	—	40	40	20	80	20	—	40	10	10	—	—	5	—	—	—	—
caprae	—	20	—	—	5	—	—	—	5	—	—	—	—	—	40	—	—
cuniculi	40	80	40	40	160	40	—	40	80	40	—	—	20	—	—	—	20
minima	—	20	10	40	40	—	—	—	5	—	—	—	—	—	—	—	—
paraffinae	—	20	5	—	20	—	—	5	10	—	—	—	10	—	—	—	—
polychromogenes	—	40	20	10	80	—	5	5	40	10	—	10	—	10	—	—	—
brasiliensis strains:																	
Ochoa 409	*80*	80	20	10	—	20	—	—	—	10	5	—	—	—	10	—	20
2178	—	*160*	20	—	20	10	—	—	10	10	—	—	—	—	—	—	40
Ochoa 414	80	—	*80*	20	—	20	—	—	5	10							
47296	20	20	10	*40*	40	5	—	—	—	5	—	—	—	—	5	—	5
992	—	160	20	—	*160*	20	—	—	—	10	—	—	—	—	20	—	20
Ochoa 413	80	160	80	—	40	*80*	—	—	—	5	—	10	5	—	—	—	—
corallina	5	80	40	20	80	10	*80*	40	160	80	5	20	20	20	20	10	80
erythropolis	10	160	20	20	80	40	80	40	160	20	10	20	40	20	—	10	20
globerula	—	80	40	20	160	10	40	*80*	80	10	—	—	10	10	—	—	—
opaca	5	80	40	40	80	—	40	20	40	10	10	20	40	80	—	—	—
rubra—NRRL B685	—	20	5	20	40	—	—	—	*80*	10	—	—	40	—	5	10	—
rubra-379	—	160	20	20	80	10	40	20	80	*160*	10	—	80	80	5	—	5
intracellularis	—	—	—	—	—	—	—	—	—	—	*320*	—	—	—	—	—	—
Streptomyces-like Nocardia[†]	—	—	5	10	10	—	—	—	—	—	—	—	—	—	—	—	—
Streptomyces griseus	—	—	—	5	—	—	—	—	5	5	—	—	—	—	—	—	—
ruber	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mycobacterium butyricum	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—
fortuitum—ATCC 6841	5	40	20	10	40	20	—	—	20	40	5	*40*	5	—	5	—	5
fortuitum—NCTC 8697	10	—	10	10	80	20	—	—	20	80	5	20	5	—	—	—	—
fortuitum—R389	—	40	20	5	80	10	10	—	20	10	5	—	5	—	—	5	—
phlei—R5	—	40	5	—	80	40	—	—	10	80	—	—	80	—	—	—	—
phlei—W23	—	—	—	—	—	—	—	—	—	5	—	—	40	—	—	—	—
smegmatis[‡]—270	—	—	—	—	—	—	—	—	—	—	—	10	—	10	—	—	—

TABLE 2—*Continued*

Antigens from Strains of:	Antisera vs. Nocardia: *N. brasiliensis* Ochoa 409	*N. brasiliensis* 2178	*N. brasiliensis* Ochoa 414	*N. brasiliensis* 47296	*N. brasiliensis* 992	*N. brasiliensis* Ochoa 413	*N. corallina*	*N. globerula*	*N. rubra* NRRL B685	*N. rubra* 379	*N. intracellularis*	Mycobacterium: *M. fortuitum* ATCC 6841	*M. phlei* 125	*M. smegmatis* NCTC 8150	*M. tuberculosis* avian—4017.2	*M. tuberculosis* bovine—4228.4	*M. tuberculosis* human—H37Rv
smegmatis‡—NCTC 8150	—	—	—	—	—	—	—	—	—	—	—	—	—	*40*	—	—	—
tuberculosis (avian)—210	—	—	—	—	—	—	—	—	—	—	—						
tuberculosis (avian)—4017-2	—	—	—	—	—	—	—	—	—	10	10	—	20	—	—	—	—
tuberculosis (bovine)—Ravenel	—	20	—	—	—	—	—	—	—	10	5	40	20	80	—	10	40
tuberculosis (bovine)—4228-4	—	40	10	—	—	—	10	—	5	10	10	80	40	40	—	*40*	20
tuberculosis (human)§—H37Rv	—	5	—	—	—	—	—	—	—	—	—	20	10	20	—	—	*40*
tuberculosis (human)—Erdman	—	—	—	—	—	—	—	—	—	5	5	20	5	—	—	10	20

*· †· ‡· § See footnotes, table 1.

antisera vs. members of the *N. asteroides* group, although the antisera to the former 4 species showed a pattern of reactivity similar to that exhibited by the anti-*N. asteroides* sera. Cell suspensions of strains from the *N. corallina* group (including *N. erythropolis* and *N. globerula*) and 2 of 3 strains representative of the *N. opaca* group showed cross reactions with most antisera vs. members of the *N. asteroides* group, whereas *N. intracellularis* failed to agglutinate with any of these antisera. Reactions were also very infrequent with suspensions from strains of *M. butyricum*, *M. phlei*, and *M. smegmatis*, as well as the nonacid-fast, nonfragmenting, streptomyces-like nocardiae and the 2 species recognized as authentic Streptomyces. Cross reactions with the various antisera were somewhat more frequently obtained with the *M. fortuitum* strains than with *M. tuberculosis* strains (avian, bovine, or human).

Tests with antisera vs. N. brasiliensis: As may be seen from table 2, the performance of *N. brasiliensis* strains and antisera suggested some antigenic heterogeneity, as was evident with the *N. asteroides* system; some of the former sera with homologous titers of 1:80–1:160 failed entirely to clump certain heterologous strains of *N. brasiliensis*. Antisera for 5 of the 6 strains of *N. brasiliensis* agglutinated most *N. asteroides* antigens about as well as they did suspensions from nonhomologous strains of their own species. In general, *N. brasiliensis* antisera behaved in a fashion similar to *N. asteroides* antisera when tested against other species of acid-fast fragmenting Nocardia, mycobacteria (except for a lesser degree of cross reaction with *M. tuberculosis* strains), Streptomyces, and the streptomyces-like nocardiae.

Tests with antisera vs. members of N. corallina group, N. opaca group, and N. intracellularis: Sera from rabbits immunized with strains of *N. corallina*, *N. globerula*, and *N. rubra* all showed patterns of reactivity (table 2) which, although by no means identical, were basically comparable to those obtained with antisera prepared against strains of the *N. asteroides* group.

The anti-*N. intracellularis* serum, in contrast, proved to be relatively specific, giving reactions only exceptionally with strains of the *N. asteroides* and *N. brasiliensis* groups, and clumping at

TABLE 3

SLIDE AGGLUTINATION TITERS IN TESTS WITH ANTISERA VS. "STREPTOMYCES-LIKE NOCARDIA" AND VS. STREPTOMYCES SPECIES, RESPECTIVELY

Antigens from Strains of:	Antisera vs. Streptomyces-like Nocardia								Antisera vs. Streptomyces	
			N. madurae			*N. pelletieri*				
	caviae	*leishmanii*	Ochoa 415	Aldana 339	ATCC 6245	Lacaz 293	47293	*rangoonensis*	*griseus*	*ruber*
Nocardia										
asteroides strains*:	—	—	10	5	—	—	—	—	5	10
brasiliensis strains†:	—	—	—	—	—	—	—	—	—	—
other "true" Nocardia species‡:	—	—	—	—	—	—	—	—	—	—
Streptomyces-like Nocardia:										
N. gardneri	—	—	5	—	—	—	—	—	—	—
N. leishmanii	320	*640*	80	—	40	20	40	80	320	160
N. madurae—Ochoa 415	—	—	*80*	5	—	—	20	—	—	—
N. madurae—Aldana 339	—	—	40	*80*	—	10	40	5	5	—
N. madurae—ATCC 6245	80	80	5	5	*160*	—	20	—	5	—
N. pelletieri—Lacaz 293	—	—	20	20	—	*20*	40	—	—	—
N. pelletieri—47293	—	—	20	5	—	20	*80*	5	5	—
N. rangoonensis	—	—	—	—	—	5	20	*20*	—	—
Streptomyces										
griseus	—	—	—	—	—	5	20	10	*40*	10
ruber	—	—	—	—	—	—	—	—	10	*80*
Mycobacterium species§	—	—	—	—	—	—	—	—	—	—

* All *N. asteroides* strains listed in table 1, including those mentioned in the footnote. The figures in the table indicate the maximal titers exhibited by a few cross-reacting strains.

† All *N. brasiliensis* strains listed in table 1. No cross reactions.

‡ All Nocardia strains listed in table 1 other than *N. asteroides*, *N. brasiliensis*, and the "Streptomyces-like Nocardia." No cross reactions observed except for *N. rubra* 379 and *N. opaca*, both of which reacted in low titer with 50 per cent of the antisera.

§ All strains of Mycobacterium listed in table 1, including those mentioned in the footnote. No cross reactions.

Italicized figures indicate the homologous titers.

low titers of some representatives of the *N. corallina* and *N. opaca* groups, as well as strains of *M. fortuitum* and *M. tuberculosis*.

Tests with antisera vs. mycobacteria: With the antisera prepared against strains of mycobacteria, titers of more than minimal level were usually only obtained with a minority of strains from the *N. asteroides* and *N. brasiliensis* groups. There appeared to be greater cross reactivity of suspensions from the *N. corallina* and *N. opaca* groups with antisera vs. saprophytic mycobacteria; the single antisera vs. the bovine and avian bacilli seemed somewhat less cross reactive.

Tests with antisera vs. Streptomyces species and streptomyces-like Nocardia: The lack of cross reactivity of antimycobacterial sera in the presence of suspensions of Streptomyces and streptomyces-like Nocardia (table 2) was paralleled by the failure of antisera vs. strains of the latter two groups to clump mycobacteria and by the low-titer, infrequent reactions with members of the "true Nocardia" (table 3).

While there was evidence of significant cross reactivity with reagents of *N. caviae*, *N. leishmanii*, and *N. madurae* ATCC 6245, these strains showed less serologic relationship to *N. madurae* strains Aldana 339 and Ochoa 415. The 2 *N. pelletieri* antisera gave reactions with *S. griseus* and some of the streptomyces-like Nocardia. *S. griseus* and *S. ruber* could be distinguished from one another by the minor degree of cross reactivity; antisera to both gave high titers in the presence of *N. leishmanii* but little, if any, reaction to other streptomyces-like Nocardia.

Discussion

Because of the large number of species examined and the limitations of the facilities available, it was not feasible in this study to immunize several rabbits for each individual microbial strain. Under these circumstances, one might ask whether the results, which are derived largely from tests with single antiserum versus the respective strains, reflect fairly faithfully the true antigenic relationships, or whether they might sometimes represent merely the peculiar immunologic responses of individual animals. While this question cannot be answered categorically, some pertinent information is available, since pairs of rabbits were used in the preparation of antiserum to several Nocardia strains. Comparative tests showed that qualitatively the immune response was essentially equivalent in each animal receiving a given antigen, and pairs of antisera exhibited parallel heterologous cross-agglutination patterns; the reactions of only one antiserum of such pairs are therefore recorded in the tables as typical of the responses obtained.

The numerical titers given by the respective antisera were not in general relatively high when compared with other serologic systems, but this was not considered a serious drawback in the light of the other advantages of the technique used. In preliminary experiments, both capillary and test-tube agglutination methods were tried but found less satisfactory in terms of one or more of the requirements for the numerous checkerboard-type titrations: elimination of confusing reactions in control mixtures without antiserum; volumes of reagents required; ease of setting up and reading the tests; and reproducibility of results in repeated trials.

Previous workers (3, 11) who have attempted to utilize the slide agglutination method for studying serologic relationships among acid-fast microorganisms, including Nocardia, considered it of only limited value because of one or more of the following: difficulty in preparing stable microbial suspensions; clumping of the latter by normal preimmunization sera (of rabbits or guinea pigs) in dilutions up to 1:10; low titers of the antisera obtained and their failure to distinguish sharply among strains. In the hands of the present writers, preparation of stable suspensions was readily achieved by the addition of sodium taurocholate in the suspending medium, following grinding with Celite. Some abrasive particles of extremely small size were observed to remain in suspensions after the mild centrifugation used to throw down large microbial clumps. This made optical standardization of the suspensions only approximate with respect to the number of cell particles per milliliter, since the ratio of Celite particles to microbial elements would be expected to vary somewhat among the different preparations. Despite the content of microscopic silica particles, the antigen suspensions were found to be surprisingly nontoxic on introduction into the circulatory system of the rabbits undergoing immunization. The antisera from such animals did not agglutinate a suspension of Celite alone, nor did preimmunization bleedings clump the antigens even in the low dilution of 1:5. It may be mentioned that, although rabbit serum proved quite satisfactory in this regard, "normal" chicken serum was found frequently to clump various of the antigens tested. The use of buffered saline (pH 7.2 to 7.4) for diluting the antigens and antisera, together with experiments designed especially to check the point, also ruled out any likelihood that acid agglutination played a role in the results.

The homologous titer of the respective antisera was usually 1:80 or higher, the test conditions making it feasible to detect titers at least sixteen fold lower; reactions with heterologous strains at titers at least one-eighth of those of the homologous system were regarded as indicative of significant serologic cross reactivity. It was noted that some antisera clumped heterologous antigens at an even higher titer than the homologous antigen, but this was not unanticipated in the light of possibly complex assortments of antigenic components and their surface distribution as well as relative reactivity, the physicochemical effects of other surface components, varying size and charge of microbial particles, et cetera. The possibility that such modifying factors might be operating was considered in the interpretation of data.

While *N. asteroides* suspensions generally showed marked reciprocal cross reactions with antisera vs. most strains of this species, indicating close antigenic relationships, the collection tested was evidently not antigenically homogeneous. There was some suggestion of unilateral cross reactivity, with certain antisera clumping heterologous *N. asteroides* strains only in low titers or not at all; two strains (CDC 144 and 359) ap-

peared to show very little mutual cross reactivity. It was of interest that the antisera and cellular suspensions prepared for two strains (356 and NRRL B970) which were reported as having been isolated originally from a dog and a cow, respectively, reacted in a manner wholly comparable to that of the corresponding reagents from strains of *N. asteroides* recovered from human sources. *N. blackwellii* and *N. cuniculi* were indistinguishable from typical *N. asteroides* strains in their serologic (as well as cultural) behavior; they should probably be reduced to synonymy with the latter species. On the other hand, the situation with regard to *N. caprae*, *N. minima*, *N. paraffinae*, and *N. polychromogenes* is less clear-cut. Antisera vs. *N. asteroides* strains only infrequently clumped suspensions of these organisms at significant titers, although antisera vs. the latter exhibited a pattern of reactivity quite like that of *N. asteroides* antisera. The older literature records analogous findings (2, 4). It might be desirable to await further work before attempting to classify the 4 species mentioned as synonymous with, or separate from, *N. asteroides* (*cf.* 12).

Although there were individual differences among strains, the pattern of serologic reactivity of *N. brasiliensis* suspensions seemed significantly different from that of *N. asteroides* (*cf.* 9); several *N. brasiliensis* antisera did, however, readily agglutinate most *N. asteroides* suspensions. A recent report largely confirms the validity of the previously reported cultural differentiation of these two species, including such criteria as thermal tolerance and ability to attack gelatin (*cf.* 10, 13), except that Gordon and Mihm (14) found that about 33 per cent of the *N. asteroides* strains studied hydrolyzed gelatin when tested by Frazier's method (15) of precipitation with $HgCl_2$. In unpublished studies from this laboratory it has been noted that many strains of actinomycetes fail to liquefy gelatin even though they are capable of giving a positive reaction in gelatin-agar plates, just as Frazier originally observed with various bacteria. Thus, of 60 strains of *N. asteroides* tested, only one liquefied gelatin, whereas all 40 strains of *N. brasiliensis* studied did so.

The behavior of the strains in the *N. corallina* and *N. opaca* groups is of special interest. They produce a soft bacterium-like growth, consisting almost entirely of bacillary forms with well-defined mycelial elements seen only on young (especially slide) cultures, although they do resemble other Nocardia in forming a submerged growth in solid media (10, 16, 17). They never show the chalky or fuzzy surface, resulting from production of a vertically erect mycelium, seen with strains of *N. asteroides* and *N. brasiliensis*, particularly on primary isolation from clinical material. Thus, they would appear to occupy a position intermediate between the latter two groups, on the one hand, and the saprophytic mycobacteria, on the other. Gordon and Mihm (12) have reclassified such intermediate strains as a single species, *Mycobacterium rhodochrous*. Wayne, Juarez, and Nichols (18), however, found their strains of *M. rhodochrous* incapable of elaborating aryl sulfatase, whereas all other species of mycobacteria tested produced this enzyme. Huppert, Wayne, and Juarez (19) also noted that, while both rapidly growing saprophytic mycobacteria and Nocardia species (including *N. rubra*) gave negative reactions in a microcolonial neutral-red test, in contrast to positive results with slowly growing pathogenic and/or unclassified mycobacteria, only the Nocardia strains produced multiple-branching filaments in slide cultures.

In the tests in this laboratory, antisera to *N. corallina*, *N. globerula*, and *N. rubra* did not exhibit significant cross agglutination with mycobacterial antigens, with the exception of *M. fortuitum* and certain *M. phlei* strains which were clumped by antisera to *M. rubra*. The data in the present study suggest that the "soft Nocardia" strains studied are perhaps more closely related antigenically to other members of the genus Nocardia than to Mycobacterium, but it is readily admitted that no far reaching conclusions are warranted from the use of small numbers of strains and unabsorbed sera; further work on this point is in progress.

The strain of *N. intracellularis* studied, which had been observed earlier to resemble mycobacteria in being strongly acid fast and in producing no filamentous branching growth, proved to be quite distinctive antigenically. Suspensions of the organism did not react with a single heterologous antiserum of the many tested, although evoking the formation of antibodies with relatively high homologous titer. Moreover, reactions of *N. intracellularis* antiserum with heterologous suspensions (whether of Nocardia, Mycobac-

terium, or Streptomyces) were very infrequent and of insignificant degree. These findings, together with the biologic properties of the strain as originally described and as studied more recently (10, 20, 21), emphasize the rather unusual properties of *N. intracellularis*; however, Shepard (22) has reported that in cultures with HeLa cells this strain grows in a manner indistinguishable from "Battey" strains of mycobacteria. Also among certain population groups, culture filtrates from *N. intracellularis* and "Battey" strains have been found to give similarly greater cutaneous reactions than are evoked by standard PPD prepared from tubercle bacilli (23).

The group previously designated as the streptomyces-like Nocardia (10) includes *N. caviae*, *N. gardneri*, *N. leishmanii*, *N. madurae*, *N. pelletieri*, and *N. rangoonensis*. These strains are completely nonacid fast; they do not fragment spontaneously into bacillary or coccoid forms; they also exhibit biochemical activities usually associated with Streptomyces species. In the past few years *N. madurae* and *N. pelletieri* have been reclassified by some workers (9, 24–27) as authentic members of the genus Streptomyces on the basis of various characteristics, including evidence of sporulation. While Gordon and Mihm (12, 28) have latterly also observed beadlike chains of spores formed by some strains of *N. asteroides*, it has long been recognized that Streptomyces species may readily undergo degenerative variation and fail to sporulate on certain artificial media, so that reliance on the presence or absence of sporulation as chief criteria for separation of genera may be somewhat hazardous (*cf.* 29). On the other hand, several groups of investigators (30–33) have recently demonstrated consistent and significant differences between Streptomyces and Nocardia with regard to chemical composition of the cell wall, and have suggested that it would be of value to extend the use of such methods as a taxonomic aid. By this means, for example, *N. gardneri* strain 6531 which had been identified with *N. asteroides* (12) was found to be quite different from other Nocardia and was reclassified as a Streptomyces (31). Again, similarities in composition of the cell walls were observed (32) between *N. asteroides* and strain 658 received as *N. leishmanii*, although the latter species has been regarded as closely related to *N. madurae* (34).

In the present experiments, the streptomyces-like Nocardia exhibited considerable degrees of individual serologic specificity and very little, if any, relationship to the acid-fast, fragmenting nocardiae or to the mycobacteria. However, the evident antigenic heterogeneity among *N. madurae* strains was in contrast to the similarities in behavior of two *N. pelletieri* strains; *N. leishmanii* appeared to possess at least one antigenic component which was shared somewhat more broadly since suspensions of this organism were readily agglutinated by antisera vs. *N. pelletieri*, *N. rangoonensis*, *S. griseus*, and *S. ruber*. There were marked serologic cross reactions among the culturally related *N. caviae*, *N. leishmanii*, and *N. madurae* strain 6245; but their antisera did not clump other streptomyces-like Nocardia or Streptomyces.

On investigating Streptomyces with the aid of serologic techniques, other workers (8, 35–38) have concluded that: (*a*) while cross reactions may be seen between members of the same or different species, it is often easy to distinguish individual strains, particularly if the antisera have been previously absorbed; (*b*) cross reactions with mycobacteria or "true Nocardia" are relatively infrequent and of low degree. The pattern of serologic reactivity which was observed for *S. griseus* and *S. ruber* was in conformity with the foregoing conclusions, and lent support to the idea that many of the strains of streptomyces-like Nocardia examined in this study might be reclassified as members of the genus Streptomyces. It may be mentioned here that in their study of phages (isolated from soil) and capable of attacking Streptomyces species or streptomyces-like Nocardia, Bradley and Anderson (39) observed various degrees of reciprocal susceptibility of the respective microbial strains; however, the few partially acid-fast strains of Nocardia and Mycobacterium tested were resistant to all of these phages.

In the light of the extensive literature regarding antigenic relationships of mycobacteria, there is perhaps little novel in the present data on this subject, but some of the findings seem worthy of brief comment, as follows. By slide agglutination, cell suspensions of *M. fortuitum*, *M. phlei*, and *M. smegmatis* were distinguishable from one another and from tubercle bacilli of warm-blooded animals (*cf.* 40–43); the 3 strains of *M. fortuitum* were evidently not antigenically homogeneous.

M. phlei and *M. smegmatis*, like strains of avian *M. tuberculosis*, reacted with antinocardial sera less frequently than did strains of *M. fortuitum*. Both bovine and human strains of tubercle bacilli were sometimes agglutinated to fairly high titers by antisera vs. saprophytic mycobacteria or various strains of the "true" Nocardia (*cf.* 4, 6, 7).

As a result of the work presented here, the writers have become convinced of the value of serologic studies as a tool for the exploration of relationships among Streptomyces, Nocardia, and Mycobacterium species—a tool which up to now has not been adequately utilized (*cf.* 44). In such studies it is obviously important to use, whenever feasible, multiple strains of a given species if one wishes to obtain an accurate picture of the range and character of antigenic behavior among the Actinomycetales. Despite the evidence of intergrading and other complexities, certain broad features of the inter-relationships have already begun to be definable. It is expected that future work, employing reciprocal cross-agglutination tests with absorbed sera, will shed additional light on the affinities and taxonomic status of the groups of microorganisms examined in this survey.

Summary

The antigenic behavior of a collection of strains including numerous representatives of Nocardia, Mycobacterium, and two species of Streptomyces was examined by means of slide-agglutination tests with antiserum prepared in rabbits.

When members of the *N. asteroides*, *N. brasiliensis*, *N. corallina*, and *N. opaca* groups were compared, they were found to behave in a generally similar manner. Strains of a given species sometimes showed antigenic homogeneity, but frequently significant cross reactivity with antiserum was exhibited to other strains and species within these groups. The members of these groups exhibited various degrees of cross reactivity with antimycobacterial serum, but were only very rarely, if at all, affected by antiserum vs. Streptomyces or the "streptomyces-like" Nocardia.

The "streptomyces-like" Nocardia, and the two species of Streptomyces tested exhibited greater evidence of antigenic individuality although some cross reactions were observed. These strains showed little, if any, reactivity with the fragmenting Nocardia or Mycobacterium species.

Mycobacteria such as *M. fortuitum*, *M. phlei*, *M. smegmatis*, and *M. tuberculosis*, while manifesting distinctive serologic properties, did sometimes react significantly with antiserum vs. fragmenting Nocardia but not with antiserum vs. "streptomyces-like" Nocardia or Streptomyces species.

Aside from evidence of the distinctiveness and/or cross reactivity of strains and species within the respective groups, the results of the study have emphasized anew the relative lack of relationships, demonstrable also by other means, between the genera Nocardia and Mycobacterium, on the one hand, and Streptomyces (including here the so-called "streptomyces-like" Nocardia), on the other.

Sumario

Estudios sobre las Nocardias y Otros Actinomicetales. II. Relación Antigénica Revelada por las Pruebas de Aglutinación en Laminilla

Se estudió el comportamiento antigénico de una colección de cepas que comprendían numerosos representantes de *Nocardia*, *Mycobacterium* y dos especies de *Streptomyces*, por medio de prueba de aglutinación en laminilla con antisuero preparado en conejos.

Cuando se compararon miembros de grupos de *N. asteroides*, *N. brasiliensis*, *N. corallina* y *N. opaca*, se observó que se comportaban en general en forma semejante. Las cepas de una especie dada revelaban algunas veces homogeneidad antigénica, pero frecuentemente mostraban destacada actividad cruzada con el antisuero a otras cepas y especies de dichos grupos. Los miembros de éstos manifestaban distintos grados de reactividad cruzada con el suero antibacteriano, pero muy rara vez o nunca los afectaba el antisuero contra el estreptomiceto o las nocardias "estreptomicetoideas."

Las nocardias "estreptomicetoideas" y las dos especies de estreptomiceto manifestaron mayores signos de individualidad antigénica, aunque se observaron algunas reacciones cruzadas. Estas cepas mostraron poca o ninguna reactividad con las especies de nocardias o micobacterias en vías de fragmentación.

Aunque manifestando distintivas propiedades serológicas, las micobacterias, tales como el *M. fortuitum*, el *M. phlei*, el *M. smegmatis* y el *M. tuberculosis*, sí reaccionaron algunas veces en forma significativa con el antisuero contra las nocardias en fragmentación, pero no con el antisuero contra las nocardias "estreptomicetoideas" o las especies de estreptomicetos.

Aparte de los signos de diferenciación y/o re-

actividad cruzada de las cepas y las especies dentro de los grupos respectivos, los resultados de este estudio recalcan nuevamente la relativa falta de relaciones, demostrable también de otros modos, entre los géneros *Nocardia* y *Mycobacterium* por un lado, y *Streptomyces* (comprendiendo las nocardias "estreptomicetoideas") por el otro.

Resume

Etudes de Nocardia et d'autres Actinomycetales. II. Rapports antigèniques montrés par les réactions d'agglutination sur plaque

Le comportement antigènique d'une collection de souches comprenant de nombreux spécimens de Nocardia, de Mycobacterium et deux espèces de Streptomyces fut examiné au moyen de réactions d'agglutination sur plaque, avec un antisérum préparé chez des lapins.

Quand les auteurs comparèrent des spécimens de *N. asteroides*, *N. brasiliensis*, *N. corallina*, et *N. opaca*, ils trouvèrent leur comportement généralement semblable. Des souches d'une espèce donnée montrèrent quelquefois une homogénéité antigènique, mais elles montrèrent fréquemment une cross-réactivité d'antisérum avec d'autres souches et d'autres espèces appartenant à ces groupes. Les membres de ces groupes montrèrent différents degrés de cross-réactivité vis à vis du sérum antibactérien, mais ne furent seulement que trés rarement, ou même pas du tout, interessés par l'antisérum contre Streptomyces ou Nocardia "pseudo-streptomyces."

Nocardia "pseudo-streptomyces" et les deux espèces de Streptomyces montrèrent une évidence plus nette d'individualité antigènique, bien que quelques cross-réactions furent observées. Ces souches montrèrent peu ou pas du tout de réactivité vis à vis de Nocardia "à fragments" ou de l'espèce Mycobacterium.

Alors que des mycobactéries telles que *M. fortuitum*, *M. phlei*, *M. smegmatis*, et *M. tuberculosis* montrèrent des propriétés sérologiques distinctes, elles réagirent quelquefois nettement avec l'antisérum de Nocardia "à fragments," mais pas avec l'antisérum de "pseudo-streptomyces" Nocardia ou de l'espèce Streptomyces.

A part l'évidence d'une réactivité distincte ou croisée des souches et des espèces dans certains groupes respectifs, les résultats de l'étude ont particulièrement montré à nouveau le manque relatif de rapport, que l'on peut démontrer aussi par d'autres moyens, entre les genres Nocardia et Mycobacterium d'une part, et Streptomyces (y compris ce qu'on appelle "pseudostreptomyces" Nocardia) d'autre part.

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THE EFFECT OF FILTRATION ON BIOLOGIC PRODUCTS[1, 2]

I. Influence on the Serologic Activity of Old Tuberculin

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Introduction

In a previous study (1) it was observed that unheated concentrated culture filtrates of mycobacteria prepared under similar conditions varied as sources of erythrocyte-sensitizing antigens. The reasons for the variation in content of erythrocyte-sensitizing substance were not investigated at that time although the different methods of filtration employed in obtaining the cell-free preparations were considered as possibly contributing to the loss of erythrocyte-sensitizing substance.

Interest in this alternative was stimulated by the work of Vogel (2) who found that the erythrocyte-sensitizing substance responsible for hemagglutination was removed from Candida or Saccharomyces antigen preparations when small aliquots were passed through Seitz filters. However, no loss of precipitating activity was observed. Lunde and Jacobs (3) also noted that a toxoplasma hemagglutination test antigen was lost by passage through a No. 02 Selas filter candle.

The fact that a serologic demonstration can be influenced by filtration of the antigen-containing menstruum implies that filter effects could be important in the preparation of cell-free immunizing agents.

As a model system, aliquots of Old Tuberculin (OT) have been passed through Seitz, fritted glass, or millipore filters and tested for their content of erythrocyte-sensitizing and precipitating antigens. It is anticipated that the data which have resulted from this study will have application in the preparation of cell-free antigens from the mycobacteria.

Materials and Methods

Filters: Seitz, fritted glass ultrafine, and millipore (HA porosity) types of filters were employed in the present investigation. Preliminary experiments were done with Seitz pads and millipore discs suitable for use in Swinny adapters. Subsequent filtrations were through 35-mm. diameter Seitz, 65-mm. diameter fritted glass, and 47-mm. diameter millipore filters which had, respectively, maximal areas of 9.6, 33.1, and 17.3 sq. cm.

Antigen: Trudeau Old Tuberculin (TOT), which had been especially prepared for use in hemagglutination studies, was the antigen tested. All dilutions were prepared in phosphate-buffered saline, pH 7.4.

Sera: Pooled rabbit antisera to the mycobacteria were employed in the experiments with sensitized sheep erythrocytes to ensure that the different antigen preparations were being measured against a representative antibody spectrum.

In the gel precipitin reactions, serum from individual rabbits was used, which had been found in preliminary experiments to possess gel precipitating antibody.

Hemagglutination and hemolytic tests: Sheep erythrocytes were washed at least three times with phosphate-buffered saline before sensitization with any of the filtered or unfiltered TOT preparations to be described later. One-tenth milliliter of packed, washed cells was added to 6 ml. of the appropriate TOT preparation, and sensitization was allowed to take place for two hours at 37°C. After sensitization, the erythrocytes were washed three times and resuspended in a 0.5 per cent concentration.

The hemagglutination and hemolytic titrations were carried out by drop procedures (4). In preparation for the inhibition tests, four hemagglutinating or four hemolytic units of antibody were determined by titrating serum as described, but with a drop of saline added to each tube before addition of the sensitized erythrocytes. The inhibition reactions were then run with two drops of the serum dilution equal to 4 hemagglutinating (HA) or 4 hemolytic (HL) units in each tube plus one drop of the inhibitor dilution. After one-half hour of incubation at 37°C., the tests were completed by the addition of sensitized erythrocytes and complement when indicated.

The end points of the regular titrations and inhibition reactions were read after one-half hour of incubation at 37°C. and again after overnight incubation at room temperature. The additional incubation was introduced to enhance any subtle difference among the antigens, since the comparative results are of interest in this study and not titers as such. The agglutinations were read by gentle shaking of the tubes after one minute of

[1] From the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported by research grants (E36C12 and E36C13) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

centrifugation in an International clinical model centrifuge.

All rabbit serum and guinea pig complement were appropriately adsorbed with normal sheep erythrocytes before use in the sensitized erythrocyte tests (4).

Tannic acid–treated sheep erythrocytes: In experiments in which tannic acid–treated erythrocytes were employed, the cells were treated according to the procedure of Rheins and Thurston (5). The serologic tests with these cells were conducted as described for normal sheep erythrocytes.

Gel precipitin reactions: Double-diffusion gel precipitin tests were conducted in 10-cm. glass tubes previously coated with a thin layer of agar (1). A 3-cm. column of the antigen-agar (1:1) mixture was added and above this 1 cm. of 0.5 per cent agar was layered, which constituted the reaction arena. Finally, 3 cm. of serum were added. The antigen preparations were originally 1:15 dilutions of TOT and the agar was at a 2.0 per cent concentration so that, after mixing 1:1, the final concentrations were TOT 1:30 and agar 1 per cent. All agar preparations were made in phosphate-buffered saline pH 7.4 with 1.0 M glycine added.

Results

Erythrocyte sensitization following Seitz filtration of Trudeau Old Tuberculin (TOT): After preliminary experiments had indicated that erythrocyte-sensitizing substance was removed from TOT by Seitz filtration, an experiment was performed to establish the effect on measured volumes of TOT.

Three 10-ml. aliquots of TOT 1:15 were filtered through a 35-mm. diameter Seitz pad, and 6 ml. of each filtered aliquot were used for sensitization of normal sheep erythrocytes. The 10-ml. volume was chosen to allow for wetting of the filter pad and to provide an excess of TOT 1:15 for inhibition experiments. It may be seen in table 1 that all detectable erythrocyte-sensitizing substance was removed from the first 10-ml. aliquot by Seitz filtration while the remaining aliquots were as effective in sensitizing erythrocytes as the unfiltered TOT. No influence was observed when the Seitz pad was first wetted (Experiment 2) by filtering 10 ml. of saline.

The fact that all erythrocyte-sensitizing substance was removed from the first 10 ml. is not considered to be more significant than if a consistent reduction in titer had been observed. More or less concentrated TOT would no doubt have given different results, and the choice of volume in these experiments happened to be a fortuitous one for clarity of demonstrating the filter effect.

Further attempts to alter the adsorptive capacity of the Seitz pads by copious washing of the filter with saline either before or after filtering the first aliquot of TOT 1:15 have not produced results different from those in table 1.

Inhibition of serologic reactions with TOT preparations: In order to determine if Seitz filtration removes all serologic activity from TOT 1:15 when small aliquots are filtered, portions devoid of erythrocyte-sensitizing substance were employed in inhibition reactions. To ensure that erythrocyte-sensitizing substance was absent, a 10-ml. portion of TOT 1:15 was filtered five times through previously wetted Seitz pads. Also included in this experiment was a portion of TOT 1:15 which had been adsorbed with normal sheep erythrocytes

TABLE 1

Hemolytic and Hemagglutination Titers Following Erythrocyte Sensitization with Aliquots of TOT 1:15 Passed in Succession Through a Seitz Filter

Pooled Antisera to:		Sheep Erythrocytes Sensitized with Filtered or Nonfiltered TOT 1:15							
		Experiment No. 1				Experiment No. 2*			
		Not Filtered	First 10 ml.	Second 10 ml.	Third 10 ml.	Not Filtered	First 10 ml.	Second 10 ml.	Third 10 ml.
H37Rv	HL†	128‡	—	128	64	256	—	128	128
	HA	32	—	64	32	64	—	32	64
R1Rv	HL	128	—	128	64	256	—	128	256
	HA	64	—	64	64	64	—	64	64
H37Ra	HL	32§	—	32	64§	64	—	64	64
	HA	—	—	16	—	8§	—	—	—

* In experiment No. 2, 10 ml. of buffered saline were filtered to wet pad before aliquots were filtered.
† HL = hemolytic titer; HA = hemagglutinating titer.
‡ Titers expressed as reciprocals of the serum dilutions.
§ Partial hemolysis or weak agglutination through the indicated titer.

TABLE 2

INHIBITION OF HEMOLYSIS (HL) AND HEMAGGLUTINATION (HA) BETWEEN POOLED ANTISERA* AND TRUDEAU OLD TUBERCULIN (TOT)-SENSITIZED ERYTHROCYTES

Inhibitor		Inhibitor Dilution†									
		15	30	60	120	240	480	960	1,920	3,840	7,680
TOT	HL	−	−	−	−	−	−	−	−	+	+
	HA	−	−	+	+	+	+	+	+	+	+
TOT Seitz-filtered	HL	−	−	−	−	−	−	−	±	+	+
	HA	−	−	+	+	+	+	+	+	+	+
TOT adsorbed with normal sheep cells	HL	−	−	−	−	−	−	−	±	+	+
	HA	−	−	+	+	+	+	+	+	+	+
Saline (control)	HL	+									
	HA	+									

* Pooled antisera to H37Ra and H37Rv diluted to contain 4 hemolytic units (HL) or 4 hemagglutinating units (HA).

† Expressed as reciprocal.

until it no longer contained erythrocyte-sensitizing substance.

The results of the experiment are recorded in table 2. The inhibition titers are those observed after overnight incubation at room temperature. They differed from the results seen after thirty minutes at 37°C. in that the hemagglutination inhibition was several tubes lower, while little change in hemolysis occurred. The important observation is that the results are essentially the same with the three preparations. Therefore, Seitz filtration or adsorption with normal sheep erythrocytes removed the erythrocyte-sensitizing substance from TOT without altering its capacity to inhibit the reaction between sensitized erythrocytes and antiserum.

The effect of Seitz filtration on sensitization of tanned cells: It was considered possible that TOT 1:15 which had been Seitz filtered and made devoid of erythrocyte-sensitizing substance for normal sheep erythrocytes might still sensitize tannic acid–treated sheep cells.

However, all tanned cell preparations in which Seitz-filtered TOT 1:15 has been employed as the antigen have not reacted in either hemolytic or hemagglutination tests. The nonfiltered TOT preparations have effectively sensitized tanned cells. Therefore that portion of TOT 1:15 which is not removed by Seitz filtration, but which will inhibit serologic reactions, apparently is not adsorbed to the surface of a tannic acid–modified erythrocyte.

The gel-precipitating activity of TOT following Seitz filtration or erythrocyte adsorption: Tube double-diffusion gel precipitin reactions were conducted according to the details of antigen dilution and technique which have been given under "Materials and Methods."

Initially nonfiltered or adsorbed TOT, TOT which had been filtered free of erythrocyte-sensitizing substance, and TOT which had been adsorbed free of erythrocyte-sensitizing substance with normal sheep erythrocytes were tested against four rabbit antisera. Precipitins were detected in each instance and the three antigen preparations gave equivalent reactions with a given serum in regard to the time of appearance, intensity, position, and number of lines demonstrated in gel.

Subsequently, TOT which had been filtered free of erythrocyte-sensitizing substance was adsorbed three times for ten minutes per adsorption at room temperature with normal or tanned sheep cells. The adsorptions were done in the ratio of 2 ml. of packed cells to 3 ml. of the 1:15 TOT preparation (6). The nonfiltered TOT, Seitz-filtered TOT, and the Seitz-filtered and adsorbed TOT were then tested against the four rabbit antisera previously mentioned. No differences were observed among the reactions with the different antigen preparations and a particular serum. The results were also comparable to those observed in the initial experiments with these antisera.

TABLE 3

ERYTHROCYTE SENSITIZATION WITH TRUDEAU OLD TUBERCULIN 1:15 FOLLOWING FRITTED GLASS OR MILLIPORE FILTRATION

Erythrocytes Sensitized with		37°C., 30 Minutes			Overnight Room Temperature		
		Experiment No.			Experiment No.		
		1	2	3	1	2	3
TOT 1:15 not filtered	HL	1,024*	1,024	1,024	2,048	1,024	1,024
	HA	32;64†	—	512	256	256	1,024
TOT 1:15 Seitz filtered	HL	—	—	—	—	—	—
	HA	—	—	—	—	—	—
TOT 1:15 fritted glass filtered	HL	1,024	256	1,024	2,048	1,024	2,048
	HA	—	—	—	256	32;64†	1,024
TOT 1:15 millipore filtered	HL	1,024	1,024	1,024	2,048	1,024	2,048
	HA	—	128‡	256	512	256	1,024

* Titers expressed as reciprocals of the serum dilution.
† Zone phenomenon—clearly positive at the indicated titers only.
‡ Partial hemolysis or weak agglutination through the indicated titer.

No evidence was found that removal of erythrocyte-sensitizing substance from TOT by Seitz filtration or a combination of Seitz filtration and adsorption with normal or tanned sheep erythrocytes influenced the gel-precipitating activity of the antigen. Sufficient volumes of each preparation remained so that they could be tested only for inhibition of hemolysis. Tested against the same pooled antiserum they displayed comparable inhibitory activity with that recorded in table 2 for the nonfiltered TOT.

The effect of fritted glass and millipore filters on the erythrocyte-sensitizing substance of TOT: The demonstration that erythrocyte-sensitizing substance can be removed by Seitz filtration presents the problem of selecting a filter that can be used to sterilize portions of antigen preparations without influencing their erythrocyte-sensitizing substance content and, presumably, their antigen content.

Fritted glass and millipore filters were tested by filtering 10 ml. of TOT 1:15 through an ultrafine-porosity fritted glass filter and an equal portion through a millipore (HA) filter. Six milliliters of each filtrate were used to sensitize normal sheep erythrocytes. Controls included erythrocytes sensitized with 6 ml. of a 10-ml. aliquot of TOT 1:15 which had been Seitz filtered, as well as nonfiltered TOT 1:15.

The results of several such experiments are recorded in table 3, where it can be seen that the millipore filter had the least influence on the erythrocyte-sensitizing substance of TOT. The difference between the fritted glass and millipore filters was most evident when the results were recorded after thirty minutes of incubation.

Both the fritted glass and millipore filters seem preferable to the Seitz filter. The convenience of the disposable membrane and the ease of cleaning are added advantages of the millipore filter in the sterilization of dilute or small volumes of antigen preparations.

DISCUSSION

The fact that an appreciable amount of the erythrocyte-sensitizing substance in TOT can be retained by Seitz filters does not necessarily mean that a significant amount of any other substance is so eliminated during filtration. Conversely, passage of erythrocyte-sensitizing substance by fritted glass and millipore filters does not establish that antigenic material has not been removed. However, the millipore filter is reported (7) to sterilize by surface retention of particles larger than the pore size of the filter, whereas the Seitz filter acts by adsorption (8). Therefore, it is reasonable to assume that it is less likely that soluble antigens or extremely fine particles would be removed by the millipore than by the Seitz filter.

The retention of the erythrocyte-sensitizing substance in TOT by Seitz filters indicates that it is similar to the substance derived from *Candida albicans* and *Saccharomyces cerevisiae* by Vogel

(2). Another similarity among these antigens is the independence of the precipitin activity from their erythrocyte-sensitizing substance content.

Vogel (2) demonstrated that pancreatic lipase inactivated the erythrocyte-sensitizing substance in his preparations, and from this and other experiments concluded that the Candida and Saccharomyces substance was probably a lipopolysaccharide. The lipid portion of the complex was thought to be essential for sensitization of the erythrocytes. No such characterization of the TOT–erythrocyte-sensitizing substance has been attempted. Evidence for a complex is found, however, in the unaltered inhibition activity of Seitz-filtered TOT or TOT which has been adsorbed free of erythrocyte-sensitizing substance with normal or tannic acid–treated sheep erythrocytes.

The data suggest that Seitz filtration as used in these studies may be of value as a means of separating erythrocyte-sensitizing substance from antigens effective in precipitin tests. Also, Seitz filtration might be used as a measure of the amount of destruction that occurs during the preparation of antigens, since a filtrate free of inhibitory activity would be presumptive evidence that the antigenic complex had not been destroyed. Such a use would of course be dependent upon a demonstrable direct influence of the filter on the antigen preparation as has been done with the erythrocyte-sensitizing substance in Trudeau Old Tuberculin.

Summary

Seitz filtration of Trudeau OT (TOT) removes the erythrocyte-sensitizing substance without altering the capacity of the TOT to react in gel precipitin tests or to inhibit hemagglutination or hemolytic tests. The adsorptive capacity of the Seitz filter is limited.

Millipore (HA) and fritted glass (UF) filters had only a slight effect on the erythrocyte-sensitizing-substance content of small volumes of TOT 1:15.

Sumario

El Efecto de la Filtración sobre los Productos Biológicos. I. Influjo de la Actividad Serológica de la Tuberculina Antigua

La filtración por Seitz de la TA de Trudeau (TAT) elimina la substancia eritrocito-sensibilizante sin alterar la capacidad de la TAT para reaccionar en las pruebas de precipitacion del gel o para inhibir la hemaglutinación o las pruebas hemolíticas. La capacidad adsorptiva del filtro de Seitz es limitada.

Los filtros "miliporosos" y de vidrio calcinado no ejercieron más que leve efecto sobre el contenido de substancia eritrocito-sensibilizante de pequeños volúmenes de TAT al 1:15.

Resume

Effet de la filtration sur les produits biologiques. I. Influence sur l'activité sérologique de la tuberculine vieillie

La filtration de Seitz appliquée à la tuberculine Trudeau OT (TOT) enlève la substance qui sensibilise les érythrocytes, sans altérer la propriété de la TOT de réagir dans les réactions avec les gel-précipitines ou inhiber les réactions d'hémoagglutination ou les réactions hémolytiques. La propriété d'adsorption du filtre de Seitz est limitée.

Les filtres "millipores" (HA) et ceux à base de verre pilé ont seulement un léger effet sur la quantité de substance qui sensibilise les érythrocytes contenue dans de petits volumes de TOT à 1:15.

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A DIRECT QUALITATIVE MICRO-NIACIN TEST FOR DIFFERENTIATING HUMAN TUBERCLE BACILLI FROM OTHER MYCOBACTERIA[1]

A Modified Procedure

MAURICE S. TARSHIS

(Received for publication January 7, 1960)

Introduction

It has been demonstrated that human tubercle bacilli produce considerably more niacin (nicotinic acid) than other mycobacteria (1–4). Because of their differences in niacin content, the former group of organisms can be differentiated from other acid-fast bacilli (5–16). Konno and his associates (5–11) have described procedures for determining the niacin content of mycobacteria, but their methods have not proved entirely satisfactory. Since many routine cultures do not contain sufficient growth for a satisfactory test, it is necessary to subculture the organisms. Sometimes it may require as long as five to ten weeks to obtain adequate growth, and in some instances even subculture may not yield enough growth. In addition, dyes or various constituents in the media may produce other color reactions or precipitates which make the interpretation of results difficult or impossible.

Although recent modifications in procedure (11–13, 15, 16) have helped overcome most of these difficulties, it was believed that the test could be improved further. Accordingly, an investigation was undertaken in an attempt to make the following three improvements. First, since culture material containing viable organisms is used to test for the presence of niacin, there is always the danger that infectious aerosols will be created during the procedure. Therefore, the tests must be performed under a bacteriologic hood and other safety precautions exercised. For this reason it was thought desirable to determine whether autoclaved water extracts of the organisms could be used instead of viable culture material. Second, because of the rather high cost of cyanogen bromide, its toxicity, and unpleasant pungent odor, it also was considered worth while to ascertain whether smaller amounts of this reagent, as well as aniline and the test material, could be used without significantly affecting the sensitivity of the procedure. Third, since the various culture media used for growing the test strains were found to have certain limitations, an effort was made to find a more satisfactory medium, that is, one which would produce early eugonic and uniform growth, lessen the time required to perform the test, not interfere with the interpretation of the findings, and give reproducible and consistent results. After much investigation these aims were achieved. This report describes a direct qualitative micro-niacin test which has been found useful as a screening tool for differentiating human tubercle bacilli from other mycobacteria.

Materials and Methods

Twenty strains of freshly isolated human tubercle bacilli, 2 strains of avian tubercle bacilli, 2 strains of bovine tubercle bacilli, 20 strains of unclassified acid-fast bacilli, and 5 strains of saprophytic mycobacteria were selected for study. These organisms were supplied through the courtesy of other workers, as indicated.[2]

[1] From the Medical Research Laboratory, Veterans Administration Hospital, Alexandria, Louisiana.

[2] Strains of human tubercle bacilli A-39 through A-53 were obtained from Mr. Frank Clifton, Veterans Administration Hospital, Alexandria, Louisiana; B-1 and B-2 from Dr. Anthony J. Sbarra, Harvard Medical School, Boston, Massachusetts; M-1 and M-2 from Dr. L. F. Bojalil, National University Medical School, Mexico City, Mexico; and strain H37Rv from Dr. William Steenken, Jr., Trudeau Laboratory, Saranac Lake, New York.

The Kirchberg strain of avian tubercle bacilli was obtained from Dr. George P. Kubica, Communicable Disease Center, Public Health Service, Chamblee, Georgia; and the Sheard strain from Dr. William Steenken, Jr.

The Copenhagen strain of bovine tubercle bacilli was obtained from Dr. George P. Kubica; and the Ravenel strain from Dr. William Steenken, Jr.

Strains of unclassified mycobacteria, AP-11 through AP-18, AS-16 through AS-20, and AB-1 and AB-2 were obtained from Mr. Frank Clifton; WP-1 and WP-2 from Dr. Marie L. Koch, Veterans Administration Center, Wood, Wisconsin; and

Human tubercle bacilli: The human tubercle bacilli and the unclassified mycobacteria were isolated from various clinical specimens, such as sputum, bronchial washings, gastric lavages, resected lung lesions, pleural fluids, lymph nodes, and catheterized ureteral urines. Of the 20 unclassified strains, 10 were photochromogens, 5 were scotochromogens, and 5 were Battey types. To avoid the use of patients' names and for convenience in arrangement, most of the strains were redesignated. All of the organisms were transferred to Löwenstein-Jensen (17) and Proskauer-Beck (17, 18) media to maintain stock cultures.

Media: After many trials of various liquid and solid media, two new solid media were developed which were found to meet the criteria desired. These were designated as Tween®-albumin and Tween-charcoal niacin test (TAN and TCN) media and were compared with a modified Tween-albumin (TA) solid medium which also proved satisfactory in preliminary trials. These three media were selected for final evaluation. Their formulas and methods of preparation were as follows:

Tween-albumin niacin test (TAN) medium

A. *Basal medium:*

Tween 80[2]	0.2 gm.
K_2SO_4 (reagent grade)	0.5 gm.
Mg citrate (dibasic, dry, pure)[4]	1.5 gm.
KH_2PO_4 (reagent grade)	5.0 gm.
Asparagine[5]	5.0 gm.
Agar (plain)	15.0 gm.
Glycerol (reagent grade)	20.0 ml.
Water (distilled), sufficient quantity	900.0 ml.

Final pH approximately 6.6

Preparation: All of the ingredients, except the agar, were dissolved in the water, and the solution was adjusted to pH 6.6. The agar was added and the mixture heated until the agar was dissolved. The basal medium was then sterilized for twenty minutes at 10 pounds' pressure and cooled to 56°C.

B. *Serum albumin solution:*

Albumin[6]	5.0 gm.
Dextrose (USP)	7.5 gm.
Sodium chloride (ACS)	0.85 gm.
Water (distilled)	100.0 ml.

Final pH 6.6

Preparation: The ingredients were dissolved in the water, the solution was adjusted to pH 6.6, inactivated for thirty minutes at 56°C., then sterilized by Seitz filtration.

C. *Completed medium:*

Solutions A and B were combined aseptically, mixed thoroughly, and the completed medium was dispensed into sterile 15 by 125 mm. screw-cap tubes.

Tween-charcoal niacin test (TCN) medium

Tween 80	0.2 gm.
K_2SO_4 (reagent grade)	0.5 gm.
Norit A[7]	1.0 gm.
Mg citrate (dibasic, dry, pure)	1.5 gm.
KH_2PO_4 (reagent grade)	5.0 gm.
Asparagine	5.0 gm.
Dextrose (USP)	7.5 gm.
Agar (plain)	15.0 gm.
Glycerol (reagent grade)	20.0 ml.
Water (distilled) sufficient quantity	1,000.0 ml.

Final pH approximately 6.6

Preparation: All of the ingredients, except the agar, were dissolved in the water, and the solution was adjusted to pH 6.6. The agar was added and the mixture heated until the agar was dissolved. The completed medium was then sterilized for twenty minutes at 10 pounds' pressure and dispensed into sterile 15 by 125 mm. screw-cap tubes. To obviate settling of the charcoal, the medium was mixed frequently during the period of dispensing.

Tween-albumin (TA) medium

Base[8]	1.3 gm.
Agar (plain)	3.0 gm.
Albumin[9]	20.0 ml.
Water (distilled)	180.0 ml.

Final pH approximately 6.6

Preparation: The basal medium was dissolved in the water by heating, the mixture sterilized for fifteen minutes at 15 pounds' pressure, then cooled to 45°C. The albumin was added, the ingredients were mixed thoroughly, and the completed medium was dispensed into sterile 15 by 125 mm. screw-cap tubes.

All media were checked for sterility and stored in the refrigerator before use.

Preparation of Inocula

Inocula of each test strain were prepared by the following two methods.

Method 1: Several large colonies of each strain

RB-6 through RB-8 from Mr. C. E. Smith, Battey State Hospital, Rome, Georgia.

Strains of saprophytic mycobacteria, *M. smegmatis*-a, b, *M. phlei*-a, and *M. butyricum* were obtained from Dr. George P. Kubica; and strain *M. phlei*-b from Dr. William Steenken, Jr.

[2] Microbiologically standardized. Hill Top Laboratories, Inc., Cincinnati, Ohio.

[4] No. C 67527. Amend Drug & Chemical Co., Inc., New York, New York.

[5] Bacto, microbiologically standardized, No. 0144-15. Difco Laboratories, Inc., Detroit, Michigan.

[6] Bovine albumin Fraction V, No. 2293. The Armour Laboratories, Kankakee, Illinois.

[7] Decolorizing carbon, Pfanstiel Chemical Co., Waukegan, Illinois.

[8] Bacto-Dubos broth base, No. 0385-02. Difco Laboratories, Inc.

[9] Bacto-Dubos medium albumin, No. 0309. Difco Laboratories, Inc.

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Introduction

It has been demonstrated that human tubercle bacilli produce considerably more niacin (nicotinic acid) than other mycobacteria (1–4). Because of their differences in niacin content, the former group of organisms can be differentiated from other acid-fast bacilli (5–16). Konno and his associates (5–11) have described procedures for determining the niacin content of mycobacteria, but their methods have not proved entirely satisfactory. Since many routine cultures do not contain sufficient growth for a satisfactory test, it is necessary to subculture the organisms. Sometimes it may require as long as five to ten weeks to obtain adequate growth, and in some instances even subculture may not yield enough growth. In addition, dyes or various constituents in the media may produce other color reactions or precipitates which make the interpretation of results difficult or impossible.

Although recent modifications in procedure (11–13, 15, 16) have helped overcome most of these difficulties, it was believed that the test could be improved further. Accordingly, an investigation was undertaken in an attempt to make the following three improvements. First, since culture material containing viable organisms is used to test for the presence of niacin, there is always the danger that infectious aerosols will be created during the procedure. Therefore, the tests must be performed under a bacteriologic hood and other safety precautions exercised. For this reason it was thought desirable to determine whether autoclaved water extracts of the organisms could be used instead of viable culture material. Second, because of the rather high cost of cyanogen bromide, its toxicity, and unpleasant pungent odor, it also was considered worth while to ascertain whether smaller amounts of this reagent, as well as aniline and the test material, could be used without significantly affecting the sensitivity of the procedure. Third, since the various culture media used for growing the test strains were found to have certain limitations, an effort was made to find a more satisfactory medium, that is, one which would produce early eugonic and uniform growth, lessen the time required to perform the test, not interfere with the interpretation of the findings, and give reproducible and consistent results. After much investigation these aims were achieved. This report describes a direct qualitative micro-niacin test which has been found useful as a screening tool for differentiating human tubercle bacilli from other mycobacteria.

Materials and Methods

Twenty strains of freshly isolated human tubercle bacilli, 2 strains of avian tubercle bacilli, 2 strains of bovine tubercle bacilli, 20 strains of unclassified acid-fast bacilli, and 5 strains of saprophytic mycobacteria were selected for study. These organisms were supplied through the courtesy of other workers, as indicated.[2]

[1] From the Medical Research Laboratory, Veterans Administration Hospital, Alexandria, Louisiana.

[2] Strains of human tubercle bacilli A-39 through A-53 were obtained from Mr. Frank Clifton, Veterans Administration Hospital, Alexandria, Louisiana; B-1 and B-2 from Dr. Anthony J. Sbarra, Harvard Medical School, Boston, Massachusetts; M-1 and M-2 from Dr. L. F. Bojalil, National University Medical School, Mexico City, Mexico; and strain H37Rv from Dr. William Steenken, Jr., Trudeau Laboratory, Saranac Lake, New York.

The Kirchberg strain of avian tubercle bacilli was obtained from Dr. George P. Kubica, Communicable Disease Center, Public Health Service, Chamblee, Georgia; and the Sheard strain from Dr. William Steenken, Jr.

The Copenhagen strain of bovine tubercle bacilli was obtained from Dr. George P. Kubica; and the Ravenel strain from Dr. William Steenken, Jr.

Strains of unclassified mycobacteria, AP-11 through AP-18, AS-16 through AS-20, and AB-1 and AB-2 were obtained from Mr. Frank Clifton; WP-1 and WP-2 from Dr. Marie L. Koch, Veterans Administration Center, Wood, Wisconsin; and

Human tubercle bacilli: The human tubercle bacilli and the unclassified mycobacteria were isolated from various clinical specimens, such as sputum, bronchial washings, gastric lavages, resected lung lesions, pleural fluids, lymph nodes, and catheterized ureteral urines. Of the 20 unclassified strains, 10 were photochromogens, 5 were scotochromogens, and 5 were Battey types. To avoid the use of patients' names and for convenience in arrangement, most of the strains were redesignated. All of the organisms were transferred to Löwenstein-Jensen (17) and Proskauer-Beck (17, 18) media to maintain stock cultures.

Media: After many trials of various liquid and solid media, two new solid media were developed which were found to meet the criteria desired. These were designated as Tween®-albumin and Tween-charcoal niacin test (TAN and TCN) media and were compared with a modified Tween-albumin (TA) solid medium which also proved satisfactory in preliminary trials. These three media were selected for final evaluation. Their formulas and methods of preparation were as follows:

Tween-albumin niacin test (TAN) medium

A. *Basal medium:*

Tween 80[2]	0.2 gm.
K_2SO_4 (reagent grade)	0.5 gm.
Mg citrate (dibasic, dry, pure)[4]	1.5 gm.
KH_2PO_4 (reagent grade)	5.0 gm.
Asparagine[5]	5.0 gm.
Agar (plain)	15.0 gm.
Glycerol (reagent grade)	20.0 ml.
Water (distilled), sufficient quantity	900.0 ml.

Final pH approximately 6.6

Preparation: All of the ingredients, except the agar, were dissolved in the water, and the solution was adjusted to pH 6.6. The agar was added and the mixture heated until the agar was dissolved. The basal medium was then sterilized for twenty minutes at 10 pounds' pressure and cooled to 56°C.

B. *Serum albumin solution:*

Albumin[6]	5.0 gm.
Dextrose (USP)	7.5 gm.
Sodium chloride (ACS)	0.85 gm.
Water (distilled)	100.0 ml.

Final pH 6.6

Preparation: The ingredients were dissolved in the water, the solution was adjusted to pH 6.6, inactivated for thirty minutes at 56°C., then sterilized by Seitz filtration.

C. *Completed medium:*

Solutions A and B were combined aseptically, mixed thoroughly, and the completed medium was dispensed into sterile 15 by 125 mm. screw-cap tubes.

Tween-charcoal niacin test (TCN) medium

Tween 80	0.2 gm.
K_2SO_4 (reagent grade)	0.5 gm.
Norit A[7]	1.0 gm.
Mg citrate (dibasic, dry, pure)	1.5 gm.
KH_2PO_4 (reagent grade)	5.0 gm.
Asparagine	5.0 gm.
Dextrose (USP)	7.5 gm.
Agar (plain)	15.0 gm.
Glycerol (reagent grade)	20.0 ml.
Water (distilled) sufficient quantity	1,000.0 ml.

Final pH approximately 6.6

Preparation: All of the ingredients, except the agar, were dissolved in the water, and the solution was adjusted to pH 6.6. The agar was added and the mixture heated until the agar was dissolved. The completed medium was then sterilized for twenty minutes at 10 pounds' pressure and dispensed into sterile 15 by 125 mm. screw-cap tubes. To obviate settling of the charcoal, the medium was mixed frequently during the period of dispensing.

Tween-albumin (TA) medium

Base[8]	1.3 gm.
Agar (plain)	3.0 gm.
Albumin[9]	20.0 ml.
Water (distilled)	180.0 ml.

Final pH approximately 6.6

Preparation: The basal medium was dissolved in the water by heating, the mixture sterilized for fifteen minutes at 15 pounds' pressure, then cooled to 45°C. The albumin was added, the ingredients were mixed thoroughly, and the completed medium was dispensed into sterile 15 by 125 mm. screw-cap tubes.

All media were checked for sterility and stored in the refrigerator before use.

Preparation of Inocula

Inocula of each test strain were prepared by the following two methods.

Method 1: Several large colonies of each strain

RB-6 through RB-8 from Mr. C. E. Smith, Battey State Hospital, Rome, Georgia.

Strains of saprophytic mycobacteria, *M. smegmatis*-a, b, *M. phlei*-a, and *M. butyricum* were obtained from Dr. George P. Kubica; and strain *M. phlei*-b from Dr. William Steenken, Jr.

[3] Microbiologically standardized. Hill Top Laboratories, Inc., Cincinnati, Ohio.

[4] No. C 67527. Amend Drug & Chemical Co., Inc., New York, New York.

[5] Bacto, microbiologically standardized, No. 0144-15. Difco Laboratories, Inc., Detroit, Michigan.

[6] Bovine albumin Fraction V, No. 2293. The Armour Laboratories, Kankakee, Illinois.

[7] Decolorizing carbon, Pfanstiel Chemical Co., Waukegan, Illinois.

[8] Bacto-Dubos broth base, No. 0385-02. Difco Laboratories, Inc.

[9] Bacto-Dubos medium albumin, No. 0309. Difco Laboratories, Inc.

were removed from six-week-old cultures of Löwenstein-Jensen medium and placed into 15 by 125 mm. sterile screw-cap tubes containing a small amount of fine white sand and several drops of 0.1 per cent aqueous albumin solution.[10] Uniform suspensions of the organisms were prepared by grinding them in the sand and albumin mixture with a stirring rod. The suspensions were then adjusted with the albumin solution to the No. 5 tube of the McFarland nephelometer standard (19) (equivalent to approximately 1.5×10^9 bacterial units per ml.).

Method 2: Several more colonies of each strain were removed from the cultures and inoculated into tubes of liquid Tween-albumin medium.[11] These cultures were then incubated for ten days at 37°C., after which they also were adjusted to the No. 5 tube of the McFarland standard.

Eight sets of the three media were then inoculated with 0.3 ml. aliquots of each test strain prepared by each of the two methods described above. The inoculated media were placed into the 37°C. incubator in a nearly horizontal position to permit uniform distribution of the inocula. Niacin tests were performed on the cultures after seven, ten, fourteen, and twenty-one days, respectively.

Techniques Used in Performing the Test

Preparation of water extracts: From 2 to 10 drops of distilled water were added to each culture, depending on the amount of fluid already present. The tubes were then placed in a nearly horizontal position for approximately five minutes to enable the fluid to cover the entire surface of the bacterial mass and thus permit greater extraction of niacin. After this time, 4 drops of extract from each culture were removed by means of separate 8-inch disposable plastic straws,[12] fitted with a removable rubber aspirating bulb, and placed into 10 by 100 mm. serologic tubes. The remaining extract in each culture was then removed by absorbing it into a 6-inch commercially prepared cotton-tipped applicator[13] which also was placed into a serologic tube. The extracts were then sterilized for fifteen minutes at 15 pounds' pressure and cooled to room temperature. For comparison, the niacin tests were performed by each of the following three methods.

Method 1 (micro-plate test): Two drops of extract from each strain were removed from the serologic tubes by means of separate plastic straws and placed into appropriately marked depressions of white glazed porcelain spot plates. To each extract were then added one drop of 4 per cent v/v aniline in 95 per cent ethanol and one drop of a 10 per cent w/v aqueous solution of cyanogen bromide.[14] The contents were mixed by sliding the plates back and forth in a rotary motion, care being taken to prevent spillage.

Method 2 (micro-tube test): To the remaining 2 drops of the extracts in each serologic tube also were added one drop each of the aniline and cyanogen bromide reagents, and the contents were mixed by shaking.

Method 3 (micro-swab test): To each serologic tube containing a cotton-tipped applicator with extract were added one drop each of the aniline and cyanogen bromide reagents. Immediately before adding each reagent the applicator was lifted out of the tube, then replaced and mixed with it.

All results were read in approximately two minutes after mixing the reagents with the extracts. The results of method 1 were read by means of a reflected fluorescent light source. The results of methods 2 and 3 were read against a white background and a similar light source. The results were graded arbitrarily as follows:

−	= negative, no color
D	= doubtful, questionable distinct yellow color
+S, +M, +I	= positive, distinct yellow color, reaction either slight, moderate, or intense, depending on the degree of coloration.

Controls: Tubes of uninoculated media were used routinely as controls and niacin tests were performed on the water blanks in exactly the same manner as for the inoculated media. Pigmented strains which colored the water extracts were also compared with the following 2 controls: (*1*) 2 drops of extract, one drop of analine, and one drop of water; (*2*) 2 drops of extract, one drop of cyanogen bromide, and one drop of water.

All procedures were carried out under a well-ventilated bacteriologic safety hood. Each test was repeated twice. The final readings represent average results.

[10] Crystallized bovine plasma albumin, No. 2266. The Armour Laboratories. The albumin solution was adjusted to pH 7.0, inactivated by heating for thirty minutes in a 56°C. water bath, and sterilized by Seitz filtration (20).

[11] Same formula as that used in the preparation of the solid medium.

[12] Tomac nontoxic plastic drinking straws, No. 12240. Scientific Products, Division of American Hospital Supply Corporation, Evanston, Illinois. Small amounts of the bacterial mass may be picked up while removing the extracts, but this does not interfere with the test.

[13] Hospital Specialty Company, Milwaukee, Wisconsin.

[14] Both reagents were freshly prepared before use and stored in tightly sealed colored dropper bottles at 5°C. Old or unrefrigerated reagents do not give dependable results. Dropper bottles are convenient for dispensing the test reagents. Since the droppers may vary in the amount of fluid they deliver, only those which deliver approximately the same quantities should be used. When not in use, the bottles should always be kept covered, even during the testing period. Under these conditions, reagents as old as six weeks have given satisfactory results.

Results

The data presented in table 1 summarize typical findings from each group of organisms. It may be seen that all of the human tubercle bacilli gave positive niacin tests, whereas all of the other strains, with one exception, gave negative results. The Ravenel bovine strain exhibited a slightly positive reaction after the fourteenth- and twenty-first-day periods. The greatest number of positive results as well as the most marked and consistent degrees of color reactions were obtained with the Tween-albumin niacin and the

TABLE 1

Results of a Direct Qualitative Micro-Niacin Test after Incubation of Cultures of Different Types of Mycobacteria for Various Periods on Three Test Media

Strain	Test Periods												
	7 Days			10 Days			14 Days			21 Days			
	Media												
	TAN	TCN	TA	TAN	TCN	TA	TAN	TCN	TA	TAN	TCN	TA	C
Human tubercle bacilli													
A-50	+S	+S	−	+M	+M	D	+M	+M	D	+I	+M	+S	−
A-51	+M	+S	D	+M	+S	+S	+M	+M	+S	+I	+I	+S	−
A-52	+M	+S	+S	+M	+S	+S	+M	+S	+M	+I	+I	+M	−
A-53	+M	+S	+S	+M	+S	+S	+M	+S	+M	+I	+I	+M	−
B-1	+S	−	+S	+S	+S	+S	+M	+M	+S	+I	+M	+S	−
B-2	+S	+S	−	+S	+S	D	+M	+M	+S	+M	+M	+S	−
M-1	+S	+S	+S	+S	+S	+S	+S	+S	+M	+M	+M	+M	−
M-2	+S	+S	+S	+S	+S	+S	+M	+M	+S	+I	+I	+S	−
H37Rv	+M	+S	+S	+M	+M	+S	+M	+M	+S	+I	+M	+M	−
Avian tubercle bacilli													
Kirchberg	−	−	−	−	−	−	−	−	−	−	−	−	−
Sheard	−	−	−	−	−	−	−	−	−	−	−	−	−
Bovine tubercle bacilli													
Copenhagen	−	−	−	−	−	−	−	−	−	−	−	−	−
Ravenel	−	−	−	−	−	−	+S	+S	−	+S	+S	+S	−
Unclassified mycobacteria													
Photochromogens													
AP-18	−	−	−	−	−	−	−	−	−	−	−	−	−
WP-1	−	−	−	−	−	−	−	−	−	−	−	−	−
Scotochromogens													
AS-19	−	−	−	−	−	−	−	−	−	−	−	−	−
AS-20	−	−	−	−	−	−	−	−	−	−	−	−	−
Battey	−	−	−	−	−	−	−	−	−	−	−	−	−
AB-1	−	−	−	−	−	−	−	−	−	−	−	−	−
RB-6	−	−	−	−	−	−	−	−	−	−	−	−	−
Saprophytic mycobacteria													
M. smegmatis-a	−	−	−	−	−	−	−	−	−	−	−	−	−
M. phlei-a	−	−	−	−	−	−	−	−	−	−	−	−	−

TAN = Tween-albumin niacin test medium; TCN = Tween-charcoal niacin test medium; TA = Tween-albumin medium; C = control; − = negative; D = doubtful; +S, +M, +I = slightly, moderately, and intensely positive. *See* Methods.

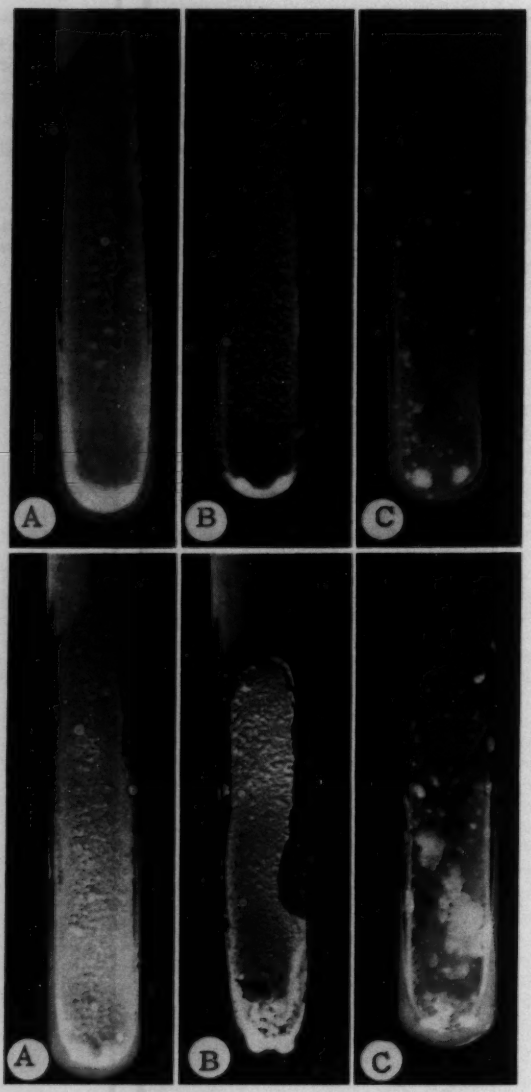

FIG. 1 (Top) and FIG. 2 (Bottom). Growth of two recently isolated human strains of *M. tuberculosis* on (*A*) Tween-albumin niacin test (TAN), (*B*) Tween-charcoal niacin test (TCN), and (*C*) Tween-albumin (TA) media after ten days of incubation at 37°C.

At this time, development on the TAN and TCN media is somewhat dryer, more eugonic, and uniform than on the TA medium. Growth on the latter medium has a tendency to be disrupted easily when handled (2*C*).

Tween-charcoal niacin media, both of which also gave earlier eugonic and uniform growth than did the Tween-albumin medium (figures 1 and 2). It may also be seen that, as the period of incubation increased, there was a corresponding increase in both the number and degree of positive results. That niacin content in strains of human tubercle bacilli increases with the age of the culture has been reported previously (11, 15).

Although each of the test procedures gave essentially similar results, those obtained with the micro-plate and micro-tube methods were somewhat easier to read than the micro-swab technique when the reactions were doubtful or slightly positive. However, when the color reactions were more pronounced, the results of all three procedures could be read equally as well.

Discussion

Although the Tween-albumin liquid medium recently recommended by Konno and Sbarra (11), as well as several modifications of this medium, were found to give positive niacin tests within a ten-day period, preliminary observations indicated that the results were never as clear-cut as those obtained with water extracts from the growth on solid forms of this and other media. Because water removes niacin not only from the bacilli, but apparently from the medium as well (15, 16), the use of water extracts rather than bacillary material appears to be more effective for performing the test.

Another limitation of the Tween-albumin liquid medium is that precipitates form when aniline and cyanogen bromide are added to it and, in some instances, the results are difficult to interpret even when controls are used. Neither can the medium be autoclaved in order to destroy viable bacilli before the test is performed since heat precipitates the albumin and this interferes with the readings.

Finally, since the yellow or orange pigments of some of the unclassified mycobacteria, especially the scotochromogens, dissolve in the liquid medium, the test under these conditions also is somewhat difficult to read even with the use of controls. This was found to occur in only two instances with highly pigmented photochromogenic and scotochromogenic unclassified mycobacteria grown on all three of the solid media studied in this investigation. However, the amount of dissolved pigment in the water extracts of these organisms was very much lighter in color than the coloration produced by the same strains in the liquid medium.

For these reasons, the Tween-albumin medium was prepared in solid form to permit water extraction of the niacin and autoclaving of the extracts.

In previous studies (21) it was observed that the Youmans' modification of Proskauer and Beck medium (17, 18) gave good niacin test results after approximately twenty-eight to thirty-five days when 1 ml. each of the aniline and cyanogen bromide reagents were added directly to the culture tubes. Somewhat better results were obtained when the medium was prepared in solid form, using 1.5 per cent plain agar to solidify the medium and water extracts of the bacterial growth to perform the tests.

To determine whether growth could be hastened and thus shorten the time before the tests could be performed, it was decided to modify the medium further by incorporating in it Tween-80 (22, 23). To neutralize any toxic effects of the Tween, 0.5 per cent bovine serum albumin (24) was added to one part of the medium, and 0.1 per cent charcoal (25, 26) to the other. The first portion was designated as Tween-albumin niacin test medium, and the second as Tween-charcoal niacin test medium.

After numerous trials, it was found that these two newly designed media not only gave more rapid, eugonic, and uniform growth during the earlier periods of development than did the Proskauer-Beck solid medium, but also more dependable niacin test results. After prolonged incubation for twenty-eight days or more, growth on the solid Proskauer and Beck medium was more abundant and, consequently, this resulted in an increasing number of positive results which compared closely with the Tween-albumin niacin, Tween-charcoal niacin, and Tween-albumin media.

Several unclassified mycobacteria, including the photochromogenic and scotochromogenic types, grew poorly or failed to grow on the Tween-albumin medium. None of the strains of any of the mycobacteria thus far tested failed to grow well on either the Tween-albumin niacin or the Tween-charcoal niacin media. The consistently best growth was observed on the Tween-albumin

niacin medium, and for this reason the earliest and best niacin test results were obtained in most instances with this medium, followed closely by the Tween-charcoal niacin medium. Because of these nutritional differences, the Tween-albumin niacin and the Tween-charcoal niacin media proved of greater usefulness than the Tween-albumin medium when performing niacin tests on a wide variety of mycobacteria. Since the success of this test depends upon the use of adequate amounts of bacillary material, it is important not only to employ media which produce early eugonic and uniform growth, but also media which give reproducible and consistent results. The Tween-albumin niacin and the Tween-charcoal niacin media appear to fulfill these criteria more adequately than the other media thus far tested.

Since the niacin content of human tubercle bacilli increases with the age of the culture, strains exhibiting negative or doubtful reactions when tested early should be incubated for longer periods before final results are recorded.

The use of disposable plastic straws and cotton-tipped applicators in place of serologic pipettes for removing the water extracts from the media and performing the tests saves time and expense in large-volume work. Whenever it is desired to save cultures for further use, then an aseptic technique is employed in preparing and removing the water extracts. In such instances, sterile pipettes or applicators are used in place of the straws, since the latter cannot be sterilized by autoclaving.

Although the additional step of disinfecting the water extracts before performing the tests may appear unnecessary when adequate safety equipment and precautions are employed, this part of the procedure is believed justifiable in large-volume testing as an added precaution in keeping infectious aerosols to a minimum. Sterilization of the water extracts does not interfere with the results.

For occasional testing of small numbers of strains, and when there is no need to save the cultures, the procedure may be simplified by adding sufficient distilled water to the cultures, slanting the tubes in a nearly horizontal position, allowing time for the extraction of niacin, and then adding several drops of each reagent directly to the extracts in the culture tubes. By this method, however, the reactions may not always be as clear-cut or as easily read as by the other methods described.

The use of two drops of test material and one drop of each reagent was found just as sensitive as the use of several milliliters of test material and 1-ml. quantities of each test reagent. In addition, the use of smaller amounts of reagents not only reduces the cost of performing large numbers of tests, but also helps materially to minimize the toxicity and unpleasantness of cyanogen bromide to which many workers have objected. As an additional aid in keeping its pungent odor to a minimum, several drops of 10 per cent ammonia or 5 per cent sodium hydroxide are added to the test plates or tubes after completion of the tests. This destroys the residual reagent.

Although some bovine and other strains of mycobacteria such as *M. marinum* and *M. ulcerans* may give weakly positive reactions, they should not be confused with human tubercle bacilli since these organisms can be distinguished by other differential tests and are not commonly encountered in clinical specimens.

Summary

An investigation was undertaken to determine whether the direct qualitative Konno test for niacin could be improved further, primarily from the standpoints of safety, economy, efficiency, and time required before performing the test. As a result of this study, a micro-procedure was developed in which these aims were achieved. When used under the conditions described, the test can be performed, in most instances, on cultures only seven to ten days old, thus reducing the usual testing time by approximately two to three weeks.

Two new solid test media were also developed which were found to give early, eugonic, and uniform growth as well as reproducible and consistent results. Because of these qualities, they have proved useful in performing niacin tests on a wide variety of mycobacteria. Further comparative tests of these media are in progress.

Although the niacin test has proved useful for differentiating human tubercle bacilli from other mycobacteria, it is recommended that, for greater effectiveness, the test be used in conjunction with other differential procedures.

Sumario

Micro-Niacino-Reacción Cualitativa Directa para Diferenciar los Bacilos Tuberculosos Humanos de Otras Microbacterias. Procedimiento Modificado

Se emprendió una investigación para determinar si podía aun perfeccionarse más la prueba cualitativa directa de Konno para la niacina, primordialmente desde los puntos de vista de inocuidad, economía, eficacia y tiempo requerido antes de ejecutar la prueba. Como resultado de este estudio, se elaboró un microprocedimiento que cumplía esos propósitos. Cuando se usa conforme a las condiciones descritas, puede ejecutarse la prueba, en la mayorís de los casos, en cultivos de no más de siete a diez días de edad, rebajando así en unas dos a tres semanas el tiempo habitual de comprobación.

También se elaboraron dos nuevos medios sólidos de ensayo que, según se observó, rendían una proliferación precoz, eugónica y uniforme así como resultados reproducibles y constantes. Debido a esas cualidades, han resultado útiles para verificar niacinorreacciones en una gran variedad de micobacterias. Nuevas pruebas comparativas de estos medios se hallan en camino.

Aunque la niacinorreacción ha resultado útil para diferenciar los bacilos tuberculosos humanos de otras micobacterias, se recomienda que, para mayor eficacia, se utilice la prueba junto con otros procedimientos diferenciales.

Resume

Une microréaction directe et qualitative á base de niacine pour différencier les bacilles tuberculeux humains des autres mycobactéries. Un procédé modifié

L'auteur a entrepris une investigation pour déterminer si la réaction directe, qualitative de Konno, à base de niaçine pouvait être encore améliorée, au point de vue sécurité, économie, efficacité et temps nécessaire pour faire la réaction. Il résulte de cette étude qu'un microprocédé fut développé, avec lequel ces buts furent atteints. Quand on l'employa dans les conditions ci-dessus décrites, la réaction put être faite, la plupart du temps, sur des cultures n'ayant que sept à dix jours, réduisant ainsi le temps habituel de réaction à à peu près deux à trois semaines.

Deux nouveaux milieux solides furent aussi mis au point, qui, trouva-t-on, donne de bonne heure, un développement uniforme, eugonique, ainsi que des résultats reproduisibles et tangibles. A cause de ces qualités, ils se sont montrés utiles dans la performance des réactions à la niaçine, sur une grande variété de mycobactéries. Des réactions comparées plus poussées de ces milieux sont en progrés.

Bien que la réaction à la niaçine ait été prouvée utile pour différençier les bacilles tuberculeux humains des autres mycobactéries, il est recommandé, pour plus d'efficacité, que la réaction soit utilisée en même temps que d'autres procédés différentiels.

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PRESENT-DAY CONCEPT OF REHABILITATION IN TUBERCULOSIS[1, 2]

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INTRODUCTION

Rehabilitation is defined by Webster as the act of restoring to a former status. This implies that an individual who has recovered from his illness should be returned to his former occupation. Unfortunately, the meaning of this word was modified by physicians treating tuberculosis, so that patients who made a satisfactory recovery were guided into less strenuous work, with limited hours, and under sheltered conditions. All too often there was a tendency in practice to think first of a new occupation, although many physicians point out the advantages of returning to the old job if there are no contraindications. In relatively few instances could this be done without disrupting the patient's life. The majority of patients were forced into occupations which were economically inferior or emotionally unsatisfactory. For some it meant no work, but dependency upon mate, parents, family, friends, or public assistance. The need for this cautious approach rested upon the notorious tendency of the disease to relapse and the possibly adverse effect of intrathoracic pressure changes and increased respiratory rate on a questionably stable lesion. Yet it has been pointed out that "The best way of assessing a man's ability is to let him try, because the disease and the human being are so variable that their reactions cannot be narrowly foreseen." (1).

With the advent of chemotherapy and early resectional surgery, the physician was able to observe the influence of antimicrobial therapy on the type and rapidity of healing of the tuberculous lesion. The pathology seen was different from that which occurs in natural healing. The picture of the soft, necrotic, coalescing tubercles teeming with bacilli and excavating to form cavities gave way to a rapidly fibrosing lesion with resolution of the exudative component, closure of cavities, and disappearance of active endobronchial disease. When cavities remained patent they were, for the most part, devoid of tuberculous pus, and their shaggy walls were now clean and lined by epithelial cells. Tubercle bacilli were difficult if not impossible to demonstrate on microscopy, culture, or animal inoculation. So changed was the pathology that several monographs have been published emphasizing this (2*a*, *b*). The clinical course of tuberculosis was likewise markedly changed. No longer did we see the toxic, febrile patient with the hacking, stridorous cough of bronchostenosis, copious sputum, and hoarseness. In a few weeks after the start of drug therapy even the most severely ill show marked improvement and return of strength. In most patients, sputum conversion as well as marked roentgenographic change occur within two months.

Much impressed by this new picture, the writers questioned the wisdom of maintaining the restrictive pattern of the past. If cavity closure, stable lesions on roentgenography, and noninfectious status have been achieved and maintained, is there any reason to anticipate that energy expenditure or movement of the chest cage will result in relapse? Slowly at first, but with ever increasing tempo as the safety of full activity became apparent, the program on the sixty-one-bed tuberculosis service was enlarged. By January, 1956, a formalized system of physical rehabilitation was embarked upon with the cooperation of the Department of Physical Medicine and Rehabilitation, and with the goal of returning the patient to his former job immediately upon discharge from the hospital. During the past three years, 155 consecutively admitted patients were placed on this program, and the results achieved form the basis for this report.

PLAN OF STUDY

The criteria for admission to this program are stable lesions on roentgenography, absence of cavitation (using laminagraphy in the anteroposterior and lateral views), and sputum consistently negative for tubercle bacilli on culture and microscopy. The patient is then advanced to full ambulatory status. He is required to make his own bed,

[1] From the Veterans Administration Hospital, Oakland, California.

[2] Presented in part at the annual meeting of the California Trudeau Society, San Mateo, California, April 2, 1959.

keep his bedside area clean, go to the main dining room for his meals, and is unrestricted as to his activities. If he is receiving streptomycin, he is taught to administer his own injections under the nurse's supervision. Bottles of para-aminosalicylic acid (PAS), isoniazid, and pyridoxine are supplied, which he keeps at his bedside. The patient is responsible for taking his own medication.

In the morning after breakfast he attends a session of muscle reconditioning. This physical exercise program is conducted by a trained physical therapist. The patients are started on a walking program of ⅛ to ¼ mile, slowly increasing up to one mile each morning. Arm flexion and abduction exercises using weights up to 10 pounds, body flexion, trunk twisting, stair climbing, knee bending, and bicycling are added gradually. Finally, by the second month the patient is on a full program which now consists of:

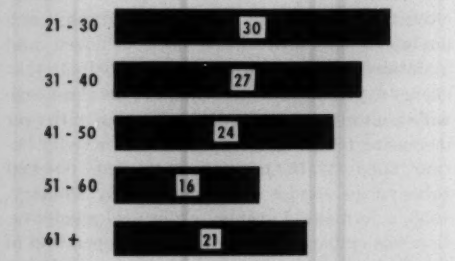
Walking—1 mile
Arm flexion and abduction with 10-pound weights, 20 times each exercise
Trunk exercises—ten minutes
Stair climbing—1 flight, round trip
Bicycle—50 repetitions
Deep knee bends—10 times each.

The occupational therapist plans a suitable work-tolerance program which will increase the muscle power and tonus of both upper and lower extremities. She prepares the patient mentally and physically for the life he will lead and the work he will do immediately upon discharge from the hospital. At the start, the patient spends one hour in the morning in the occupational therapy shop, and this is increased up to three hours daily. He is given progressively more strenuous activity and required to increase his speed of production. These objectives are accomplished by having the patient participate in a variety of skills requiring the use of hand- and foot-operated tools. He is required to stand and move around for the greater part of the therapy period, and the size and weight of materials are steadily increased. Simulated work situations are created in the clinic. Whenever possible, he is guided in the use of activities and tools which relate to his previous employment. The activities most frequently used are:

Hand sawing, hand polishing, bicycle jig saw, hand- and foot-operated printing presses, filing, floor lathes, treadle sander, sanding, large floor model looms.

Age (figure 1): The ages of the present patients ranged from twenty-one to seventy-six, with 30 patients younger than thirty, and 21 older than sixty-one years. The median age of the group was forty-one.

Extent of disease (figure 2): The disease was minimal in 27, moderately advanced in 53, and far advanced in 27. Miliary tuberculosis was present in 5 patients, and pleural effusion in 6.



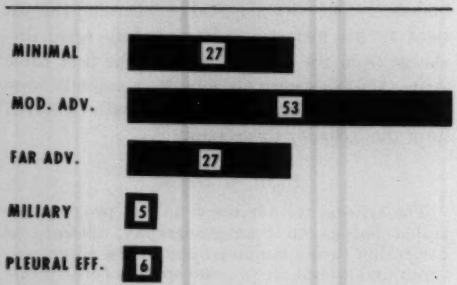


FIG. 1 (Top). Age of patients.
FIG. 2 (Bottom). Extent of disease.

RESULTS

One hundred and fifty-five male patients have been placed on this program since January 1, 1956. This group included all of the patients who had attained noninfectious status, had closed their cavities, and whose lesions were stable on roentgenography. A sizeable number participated in a necessarily limited program because of emphysema, physical defects, and old age. Information has been obtained on the post-hospital experience of 118 patients, and forms the basis for this report (figure 3).

Of this group, 88 have returned to full work activity upon discharge from the hospital. Their occupations vary from pick and shovel laborer to teacher; farm worker to agricultural inspector; construction worker and aircraft mechanic to student; warehouseman to tool and die maker. Twenty-two are not working (figure 4). Of these, 10 had been retired before they entered the hospital for the treatment of their tuberculosis. Their ages ranged from fifty-six to seventy-six, with a median age of sixty-five. Because of other limitations or a combination of disabilities, e.g., psychiatric disorders, emphysema, et cetera, 12 were unable to work. The median age for this sub-group was sixty. It is important to note that patients in the older age group, with even

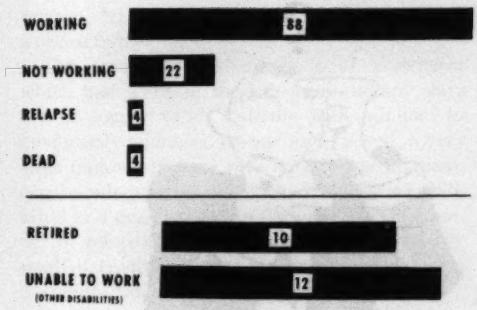


FIG. 3 (Top). Present status.
FIG. 4 (Bottom). Not working.

minor disabilities, find it increasingly difficult to obtain employment unless they had a job and a good work record at the time of hospitalization. These people suffer from the prejudices and impersonal attitudes all too prevalent in modern society.

There have been 4 deaths: one from cirrhosis of the liver (autopsy showed no evidence of active tuberculosis); one from heart disease; 2 in individuals older than sixty, both of whom were emphysematous and chronic alcoholics. It was not possible to learn the exact cause of death in these 2 patients. In none of the patients was tuberculosis mentioned as the primary or contributing cause of death.

Four patients have been or presently are hospitalized for active tuberculosis. They never did return to work: 2 of them have been arrested for drunkenness, and one is presently hospitalized in a prison ward, serving a sentence for alcoholism. These patients were alcoholics who would not accept help from Alcoholics Anonymous and who resumed their former mode of life upon discharge. The fourth patient had some slight progression of his disease roentgenographically, and his sputum became infectious. He was rehospitalized, and has since been discharged home to full activity.

The need for rest in the treatment of active pulmonary tuberculosis was universally accepted and formed the cornerstone of therapy until recently, when a questioning attitude developed as to the role rest plays in the recovery of tuberculous patients under adequate drug therapy. Hirsch and his co-workers (3), in a well-controlled study, compared the effects of short-term bed rest and physical activity in twenty-one young adult female patients. This study was conducted in a hospital where the amount of rest and degree of physical activity were carefully supervised. They demonstrated that recovery from pulmonary tuberculosis can be achieved without bed rest in individuals with recent disease caused by a drug-susceptible organism. They suggested that rest for the asymptomatic patient has little, if any, effect on the rate or degree of recovery and that more harm than good may result from prolonged inactivity in these patients. More recently, the Tuberculosis Chemotherapy Centre in Madras, India, has reported (4) a one-year study of one hundred and sixty-three patients randomized between home and sanatorium care while under chemotherapy for active pulmonary tuberculosis. Although it was difficult to evaluate activity at home versus activity in the sanatorium, the patients in the home series were physically much more active than those in the sanatorium. The percentage of the males on home care who had returned to part-time or full-time activity was 13 per cent by three months, reached 35 per cent at six months, 57 per cent at nine months, and 80 per cent at twelve months. On the other hand, only a small proportion of the male sanatorium patients were officially up for four hours a day, namely, 8 per cent at three

FIG. 5. "Taking it easy."

months and 19 per cent at twelve months. At the end of one year, 10 per cent of the females in the sanatorium were allowed up for four hours a day, whereas 91 per cent of the females on home care had returned to part-time or full-time activity. In spite of this difference in activity, the results of treatment judged on the basis of sputum "conversion," cavity closure, roentgenographic stability, and clinical findings were not statistically different in the two groups. The present study differs from the one conducted by Hirsch and that of the Madras group. The effect of activity in the early treatment of tuberculosis in hospitalized patients was not being studied, nor was a comparison made between home care and sanatorium care programs. The major interest was in increasing the muscle tone of the patients so that they could be returned to their former jobs regardless of the strenuousness of such work and to see if the relapse rate would be any greater in this group.

On the basis of this experience, it seems justifiable to urge physicians who treat tuberculosis to reexamine their ideas of rehabilitation. When doctors advise patients to "take it easy," or recommend "light work" of "four hours a day," are they not mouthing meaningless, absurd phrases? We may be approaching a thirty-five–hour work week, but not a twenty-hour one. It was the late Herman Biggs who pointed out the danger of treatment that "admits self-respecting sick men to a sanatorium and turns out healthy loafers"(5). What does one mean by "taking it easy"? Many of the patients feel that staying home, avoiding gainful occupations, frequenting bars, cross-country traveling, et cetera, is "taking it easy" (figure 5). But every expenditure of energy is work whether or not monetary compensation is involved. "For Satan finds some mischief still for idle hands to do" is certainly borne out by experience. As Clark (1), discussing the problem in England, states: "In modern conditions of life there are perhaps only two professions in which a man can work in his own time and call his soul his own—the priesthood and crime. Unfortunately the Ministry of Labor will not recognize either for their training schemes."

The causes for tuberculous relapse are not all known, but stress and alcoholism loom as the two most frequently associated factors. To attempt to make a bookkeeper out of a longshoreman (figure 6) or a watchmaker of a truck driver,

FIG. 6. Frustration.

can only lead to frustration, tension, and economic distress. Men who have worked with their hands and muscles all of their lives cannot be expected to enjoy or be capable of desk work. Perhaps under these circumstances we are reproducing the effect of administering the corticoids with the same attendant disastrous results.

* * *

Rehabilitation means different things to different people. The goal set may vary from early discharge from the hospital (medical rehabilitation) to return to a full, active, normal life (total rehabilitation). To achieve the latter, one must start the rehabilitation program the day the patient enters the hospital. He must be informed of the extent of his disease, assured of the optimistic outlook for his recovery, and advised that he will be able to return to his former occupation upon discharge. Let us not forget that, all too often, loss of confidence may be the only barrier to a return to effective work. When cavity closure or roentgenographic stability and sputum "conversion" are reached, the patient should be started on a dynamic muscle rebuilding program. If, after two months, he shows no evidence of roentgenographic or bacteriologic

relapse, he should be discharged back to full work activity.

The traditions and methods of yesteryear which had merit in the pre-chemotherapy days are no longer valid. Patients with minimal or moderately advanced disease and, indeed, a very large number of those with far advanced disease, can be adequately treated and totally rehabilitated in a period of six to ten months. The number of relapses in this group will be extremely small if cavities are no longer present, sputum is noninfectious, and chemotherapy continued.

Summary

One hundred and fifty-five consecutively admitted tuberculous patients who had achieved sputum "conversion" and cavity closure were placed on an intensive rehabilitation program geared to returning them to their former occupations. Follow-up reports have been obtained on 118 patients. Of these, 88 were fully employed in occupations varying from heavy physical labor to white collar jobs. A total of 22 were not working; these were for the most part in the older age groups, and consisted of 10 who had reached retirement age prior to hospitalization, while 12 with a median age of sixty usually had some other associated disability, such as pulmonary emphysema. Of the 4 deaths, none was related to tuberculosis. Three relapses occurred in severe alcoholics, none of whom ever attempted to return to work.

Sumario

Concepto Actual de la Rehabilitación en la Tuberculosis

A 155 tuberculosos recibidos consecutivamente que habían logrado "viraje" del esputo y cierre de las cavernas, se les impuso un plan intenso de rehabilitación, proyectado de modo que les permitiera volver a sus antiguas ocupaciones. Se han obtenido informes de observación subsecuente con respecto a 118 enfermos. De éstos, 88 trabajan a tiempo completo en oficios que varían de esfuerzo físico pesado a empleo en oficina. Un total de 22 no trabajaba; éstos pertenecían en su mayor parte a los grupos de edad avanzada, comprendiendo 10 que habían alcanzado la edad de la jubilación antes de hospitalizarse, mientras que 12 de una edad media de 60 años tenían por lo general alguna otra incapacidad, tal como enfisema pulmonar. De los 4 fallecimientos, ninguno guardaba relación con la tuberculosis. Hubo 3 recidivas en alcohólicos empedernidos, ninguno de los cuales jamás trató de trabajar de nuevo.

Resume

Conception d'aujourd'hui sur la réhabilitation dans la tuberculose

Cent cinquante cinq malades tuberculeux admis consécutivement, ayant achevé leur "conversion" d'expectoration et ayant leur caverne fermée, furent placés dans un programme de réhabilitation intensive, orienté vers le retour à leurs occupations antérieures. On put suivre les observations de 118 malades. Parmi ceux-ci, 88 furent employés complètement dans des emplois allant d'un dur travail à des emplois de bureau. Un total de 22 ne travailla pas; ceux-ci furent trouvés pour la plupart dans les groupes plus agés et comprenaient 10 malades qui avaient atteint l'âge de la retraite avant leur hospitalisation, tandis que 12, d'un âge moyen de soixante ans, avaient quelqu'autre incapacité, telle que de l'emphysème pulmonaire. Parmi les 4 morts, aucune ne fut due à la tuberculose. Trois rechutes se produisirent chez de grands alcooliques, dont aucun d'eux n'essaya même de retourner au travail.

Acknowledgments

The writers are deeply indebted to Mrs. Amabel Phares, who carefully collected the information which made this study possible; Miss Maud Greenwood for the illustrations; and Mr. Hal Strong and Mrs. Ruth Mallonee, of the Medical Illustration Service, for preparing the bar graphs.

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Case Reports

THE SERUM ELECTROPHORESIS IN SARCOIDOSIS BEFORE AND AFTER DEVELOPMENT OF TUBERCULOSIS[1]

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Introduction

This report is concerned with a patient who had sarcoidosis and later developed tuberculosis. There were several interesting aspects of the case which merit recording, such as skin reactions and, more specifically, electrophoretic patterns.

Case Report

A 33-year-old Negro female (E. J.) was admitted to the Duval Medical Center on January 23, 1959, with complaints of shortness of breath, cough, and left upper chest pain of two weeks' duration.

Present illness: The patient had a previous diagnosis of generalized sarcoidosis and had been on prednisone since June 12, 1958. The chest pain was localized in the area of a previous pectoralis muscle and skin biopsy, but had never been this severe or persistent. The cough and shortness of breath had been progressively severe over the two-week period, but the patient did not have night sweats, weight loss, or anorexia. She had had such episodes on several previous occasions.

Past history: The patient had had the usual childhood diseases, including mumps and two episodes of "double pneumonia," and at fourteen years of age she was treated for "malaria." Limitation of physical activity by dyspnea on exertion, without wheezing, was a prominent feature of her childhood but cleared in her late teens. In 1951 she developed paralysis of the left side of the face and trunk, following the delivery of her fourth child. At this time she also had enlarged masses at the angle of both jaws similar to mumps; edema of the face, hands, and feet; headache; and yellow spots before her eyes. It is not possible to substantiate this part of the history. A chest roentgenogram was reported as normal at that time, but review of the film shows bilateral hilar adenopathy. The paralysis regressed completely during the following year, and the edema and swelling of the jaws resolved rapidly. According to the patient there were three or four recurrences of the swelling of the jaws during the following seven years. A clinical examination on June 9, 1953, for complaints of cough, shortness of breath, and blood-streaked sputum revealed splenomegaly and anemia with normal vital signs.

The patient was admitted to Duval Medical Center on November 19, 1953, when examination showed no peripheral lymphadenopathy or skin lesions, and no hepatomegaly was noted. The spleen was enlarged to the level of the umbilicus and a lemon-sized mass was present in the left adnexal region; further details are not recorded. Laboratory data were as follows: the hemoglobin was 10 gm., leukocyte count 3,850 per cu. mm. with the following differential count: 50 per cent polymorphonuclear leukocytes, 49 per cent lymphocytes, and 1 per cent eosinophils. The bleeding and clotting times were normal. The sickle cell preparation was negative, the platelet count 378,000, and bromsulfalein 2.5 per cent retention in forty-five minutes. The serum bilirubin was 0.3 mg. per 100 ml.; and total protein, 9.1 gm. per 100 ml., with albumin 4.2 gm. per 100 ml., and globulin 4.86 gm. per 100 ml.

Sternal bone marrow aspiration was read as follows: myeloblasts 3, promyeloblasts 5, myelocytes 11, juveniles 3, stabs 30, segmenters 8, eosinophils 2, erythroblasts 20, and normoblasts 28. The tentative diagnosis was erythroblastic hyperplasia. The chest roentgenogram was reported as normal (that film is not now available), and the reaction to intermediate PPD was negative. The patient was thought to have Banti's syndrome, and a splenectomy was performed on December 3, 1953. The liver and abdominal lymph nodes were biopsied at surgery, and all showed epithelioid cell granulomas, scattered giant cells, and no evidence of caseation. Stains for acid-fast bacilli were negative. The patient did well postoperatively.

A chest roentgenogram obtained on February 11, 1954, showed a small nodular infiltrate in both upper lung fields, opacification of the short fissure, and bilateral hilar adenopathy. On May 20, 1954, nodules were noted on the eyelids and nose, and the patient had conjunctivitis with photophobia which was treated with opthalmic cortisone ointment. The patient was readmitted to the hospital on June 3, 1954, with uterine enlargement. The reaction to second-strength PPD was negative; the total protein, 8.4 gm. per 100 ml.; albumin, 4.8 gm. per 100 ml.; globulin, 3.6 gm. per 100 ml.; and the serum calcium, 10 mg. per 100 ml. Biopsies of the skin of the nose, chest, and chest muscle were performed. Noncaseating epithelioid

[1] From the Veterans Administration Sarcoidosis Research Unit; the Jacksonville Hospitals Educational Program, Inc.; and the Medical Service. Duval Medical Center, Jacksonville, Florida.

cell granulomas were found in the skin of the nose; the skin and muscle of the chest were normal. On June 17, 1954, the patient was given 75 mg. of cortisone three times daily, and the eye lesions soon regressed. After one week the cortisone was reduced to 25 mg. three times daily. On July 2, 1954, the patient complained of abdominal pain, and a diagnosis of pelvic inflammatory disease was made.

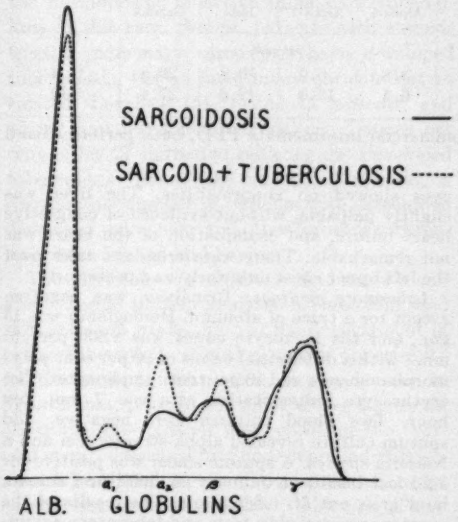

FIG. 1. The serum electrophoretic pattern before and after development of tuberculosis.

The patient was hospitalized for a third time on December 24, 1954, with chief complaint of vaginal bleeding, and had a spontaneous abortion. The cortisone dosage was reduced to 25 mg. per day. She terminated her sixth pregnancy with a normal delivery on October 16, 1955, and cortisone was discontinued on April 19, 1956. Since that time she has been employed as a practical nurse in a general hospital.

On November 25, 1956, the patient was admitted to the hospital for investigation of the left adnexal mass noted in 1953. A left salpingo-oophorectomy and appendectomy were performed. Histologically, both the ovary and the appendix revealed noncaseating granulomas with giant cells; no acid-fast bacilli were seen. She did well postoperatively, with little clinical or roentgenographic change, until January 10, 1957, when a chest roentgenogram revealed an increase in the nodular infiltration of the right upper lobe. By June 12, 1958, there was an increase in the infiltrates in both upper lobes, the patient was becoming short of breath, and noted malaise with some vague chest pains. On this date she was given 5 mg. of prednisone three times daily. Her hemoglobin was 14 gm.; the leukocyte count, 7,300 per cu. mm., and differential count was: 47 per cent polymorphonuclears, 49 per cent lymphocytes, and

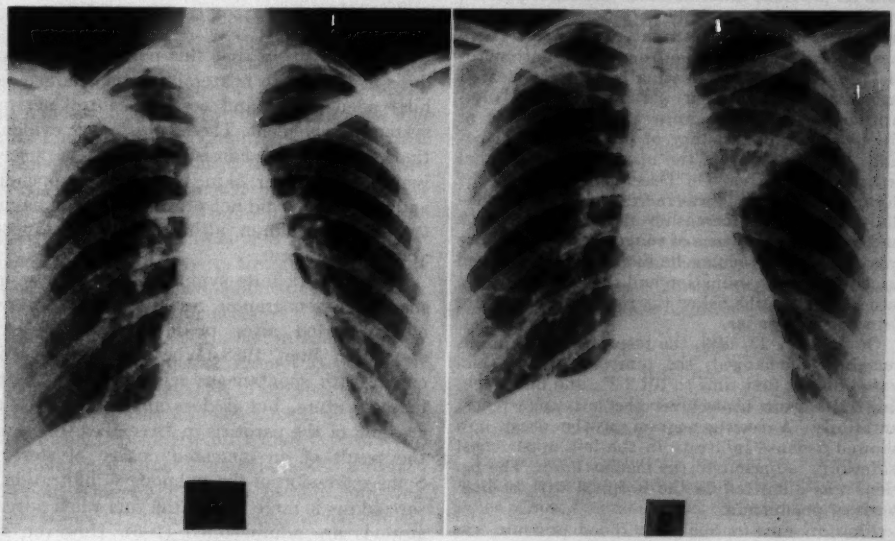

Fig. 2*A*. The postero-anterior view of the chest on November 17, 1958, showing a generalized mottling of the lung fields. *B*. The postero-anterior view of the chest on January 22, 1959, showing the additional left upper lobe infiltrate with development of tuberculosis.

TABLE 1

CORRELATION OF CHANGES IN SERUM PROTEIN VALUES WITH SKIN SENSITIVITY TO PPD (INTERMEDIATE)

Date	Total Protein	A/G Ratio	Albumin	Globulins				PPD*
				Alpha-1	Alpha-2	Beta	Gamma	
	gm. per 100 ml.		*per cent*	*per cent*				
11-18-58	8.5	0.9	51.8	3.8	6.9	10.8	26.8	2
2-4-59	8.1	0.7	40.8	6.1	13.0	12.6	27.4	30

* Millimeters of induration to freshly prepared commercial intermediate PPD, both performed and read by same physician (CFM).

4 per cent eosinophils. There was no further change in the chest film until September 30, 1958, when a slowly evolving regression of the pulmonary infiltrates began which continued until January, 1959.

On November 17, 1958, the patient was doing rather well; the prednisone had been decreased to 2.5 mg. twice daily, and the physical examination was negative except for slight enlargement of both parotid glands. Results of roentgenograms, skin test, and laboratory examination are seen in figures 1 and 2, and in table 1. On December 11, 1958, the patient complained of nausea, vomiting, itching of the eyes, and increased lacrimation. She also noted painful swelling in the region of the left parotid gland. On physical examination there was bilateral congestion, hyperemia, and granular appearance of the palpebral and bulbar conjunctivae. Both lacrimal glands were noted to be larger than on previous examinations. The right parotid was just palpable and the left parotid was considerably larger than the right, and tender. There was no ocular improvement on topical antimicrobials, and the gastrointestinal symptoms cleared on aluminum hydroxide gel and a bland diet. Anticholinergic drugs were withheld because of the patient's long history of persistent constipation. Barium study of the upper gastrointestinal tract was negative. On December 18, 1958, the dosage of prednisone was increased to 5 mg. twice daily because of increasing shortness of breath and cough. No evidence of congestive heart failure was noted. The ocular findings were unchanged from previous examination and the liver was down two fingerbreadths below the right costal margin, firm and nontender.

On January 22, 1959, the respiratory symptoms remained unchanged, the temperature was elevated for the first time to 101.2°F., and fine crackling rales were heard over the left upper chest anteriorly. A roentgenogram of the chest now showed a dense infiltrate in the left upper chest anteriorly, adjacent to the mediastinum. The patient was admitted to the hospital with a diagnosis of pneumonia.

Physical examination: The blood pressure was 110 systolic, 70 diastolic in mm. of mercury, pulse 88 per minute, respiration 30 per minute and shallow, and the temperature was elevated to 100°F. The left parotid gland was still enlarged, but the eyes showed no abnormalities. The liver was slightly palpable, without evidence of congestive heart failure, and examination of the heart was not remarkable. There were crepitant rales over the left upper chest anteriorly and posteriorly.

Laboratory findings: Urinalysis was negative except for a trace of albumin. Hemoglobin was 13 gm., and the leukocyte count was 8,800 per cu. mm., with a differential count of 90 per cent polymorphonuclears and 10 per cent lymphocytes. The erythrocyte sedimentation rate was 7 mm. per hour. Two blood cultures were negative, and sputum culture revealed alpha-streptococci and a Neiseria species. A sputum smear was positive for acid-fast bacilli on January 26, 1959, and culture later grew out *M. tuberculosis*. The results of the roentgenograms, skin test, and laboratory examinations may be seen in figures 1 and 2 and in table 1.

DISCUSSION

It is very likely that this patient had sarcoidosis in 1951, with bilateral parotid enlargement, hilar adenopathy, and possibly central nervous system involvement. There is histologic evidence that she had sarcoidosis involving the spleen, lymph nodes, liver, skin, lungs, eyes, appendix, and ovary. This did not prevent her from having two normal children, although one spontaneous abortion did occur.

There was definite symptomatic response and some roentgenographic resolution over a three-month period after prednisone therapy was started in June, 1958. Whether this was coincident with spontaneous regression is a matter for conjecture, but it does appear that the regression of the parotitis in December, 1958, was the result of an increased dosage of steroids. Some regression of the pulmonary infiltration of sarcoidosis is more or less the rule when adrenal steroids are administered (1–5). However, the roentgenographic clearing is frequently not complete even with symptomatic improvement (4), and more often is only partial in regard to both

symptoms and chest film (1–3, 6). A few cases of dramatic response of pulmonary infiltration to steroid therapy have been reported (7, 8).

Steroids may have been the causative agent in the development of active pulmonary tuberculosis in this case. Several patients with steroid-treated pulmonary sarcoidosis have developed tuberculosis, but so have many patients not so treated. Lovelock (5) treated 12 patients, and 45 per cent developed tuberculosis. Twenty per cent of his 27 untreated patients also developed tuberculosis. Before the advent of steroids, a considerable number of patients with sarcoidosis developed pulmonary and other forms of tuberculosis, both human and bovine.

The fortuitous circumstance that made available a serum protein analysis, a chest film, and skin tests ten weeks prior to the development of active tuberculosis offered a unique opportunity for this multiple comparison. It may be seen in figure 1 that, prior to the tuberculosis, the serum electrophoretic pattern was typical of the "sarcoid steps" described by Sunderman and Sunderman (9). All values were within normal range except the gamma globulin (table 1). These quantitative values might occur in many diseases, but the important finding is the scan pattern, which shows a steplike increase from alpha-1 to gamma globulin. The hyperproteinemia with a low A/G ratio, often associated with sarcoidosis, was also evident. The electrophoretic pattern following the diagnosis of active pulmonary tuberculosis showed a twofold elevation of the alpha-1 and alpha-2 globulin fractions, with a corresponding depression of the albumin. These alterations have been noted by many (10, 11) as a common feature of pulmonary tuberculosis. The gamma globulin was still elevated. The resultant electrophoretic pattern, therefore, was rather a hybrid of the two different configurations of sarcoidosis and tuberculosis.

Whether the patient still had sarcoidosis when she developed tuberculosis cannot be answered categorically. It is the belief of the present writers that the sarcoidosis was not a form of tuberculosis which later changed into a more typical form, but that there was a superimposed acid-fast infection. This is supported by the following observations: (*1*) From the change in her clinical status as related to the lungs, she must have had tuberculosis by December 18, 1958, when she rapidly became short of breath and had an increase in cough with occasional paroxysms. At this same time the patient was still having eye symptoms and some tenderness of the left parotid gland. As the parotid gland involvement had been intermittent since 1951, it is unlikely that it was caused by tuberculosis for that period of time. There was no reason to suspect that the latest episode was different from the previous ones, particularly in view of the response to steroids. (*2*) The PPD, which had given negative reactions for years during active sarcoidosis, suddenly gave a positive reaction. This would seem to indicate a response to a new disease process. (*3*) The dramatic change in serum globulins as manifested by the electrophoretic pattern is suggestive of some kind of change in antibody production, and therefore a change in antigenic stimulation.

Summary

The case of a patient who had sarcoidosis of multiple organs for at least eight years and who then developed active pulmonary tuberculosis has been presented. Associated with the change in clinical status, the tuberculin skin test became positive and the electrophoretic pattern, which had previously been compatible with sarcoidosis, promptly changed to include alterations also compatible with tuberculosis.

Sumario

La Electroforesis Sérica en la Sarcoidosis Antes y Después de la Aparición de Tuberculosis

Preséntase el caso de un sujeto que tuvo sarcoidosis de varios órganos a lo menos ocho años y luego manifestó tuberculosis pulmonar activa. Junto con la alteración del estado clínico, la cutirreacción a la tuberculina se volvió positiva y el patrón electroforético, que había sido antes compatible con sarcoidosis, varió rápidamente para comprender alteraciones compatibles con tuberculosis.

Resume

L'electrophorèse du sérum dans la sarcoïdose avant et après le développement d'une tuberculose

Les auteurs présentent le cas d'un malade qui eut une sarcoïdose intéressant de multiples organes pendant au moins huit ans, et qui développa par la suite une tuberculose pulmonaire active. Au moment de ce changement de forme clinique, la cutiréaction à la tuberculine devint positive et le traçé électrophorètique, qui avait été aupara-

vant compatible avec une sarcoïdose, changea rapidement et comprit des modifications aussi compatibles avec une tuberculose.

Acknowledgments

The writers wish to thank Dr. Max Michael, Jr., for his assistance in preparing this report.

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PULMONARY ASPERGILLOSIS WITH CAVITATION SECONDARY TO HISTOPLASMOSIS[1, 2]

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INTRODUCTION

Several species of Aspergillus are capable of causing a variety of disease patterns in man. The respiratory tract serves as the most common portal of entry for the organism. The fungus is ubiquitous and grows as a saprophyte in nature. Repeated exposure to dry particulate organic matter, such as moldy or spoiled feed, grain, hay, straw, or rotting wood contaminated with the organism, provides the most common means of infection by inhalation of spores.

Pulmonary aspergillosis with cavitation occurs as both a primary and secondary infection. This disease form has often been called pseudotuberculosis in the literature because of its clinical and roentgenographic similarities to tuberculosis. More commonly it is a secondary infection superimposed upon an antecedent pulmonary disease. The underlying lung diseases usually invaded by the Aspergillus fungus are pneumonia, cysts, fistulas, abscesses, carcinomas, infarctions, pneumoconioses, bronchiectasis, trauma, and tuberculosis. The cavitary form of aspergillosis can also be attributed to the widespread and protracted use of antimicrobials, corticosteroids, radiation, and antineoplastic drugs (1). Debilitation, diabetes, splenectomy, recent surgery, infancy, and leukopenia may also predispose to the infection. Coexistence of the Aspergillus organism with other mycotic infection of the lungs, except for candidiasis, has rarely been described. A case of lymphosarcoma associated with coccidioidomycosis, aspergillosis, and candidiasis has recently been reported (1).

The purpose of this report is to present a case of pulmonary aspergillosis with cavitation secondary to histoplasmosis. It is the first documented report of pulmonary cavitation caused by the association of these two mycotic infections and presents a challenge in differential diagnosis.

CASE REPORT

T. K., a 28-year-old single white male farmer, was admitted to the University of Chicago Clinics on September 3, 1959, with the diagnosis of chronic cavitary histoplasmosis. He had spent his entire life, except for two years of Army service, on his parents' farm in southern Illinois. The patient was first seen in August, 1943, at the age of thirteen by his family physician and was referred to the Macon County Tuberculosis Sanatorium, Decatur, Illinois, because of frequent colds and fevers over a nine-month period. There was no history of exposure to tuberculosis, and a chest roentgenogram was normal. The tuberculin skin test was negative to 0.0025 mg. of PPD in November, 1942, and in August, 1943.

The patient felt well until September, 1956, when he developed a "chest cold." This illness was characterized by moderate productive cough, prostration, fever to 102°F., and night sweats. A tentative diagnosis of viral pneumonia was made. Streptomycin and chlortetracycline were prescribed, without benefit. Because of persisting fever, the patient was hospitalized at Decatur and Macon County Hospital, Decatur, Illinois, on September 23, 1956. His weight was 136 pounds. The physical examination was essentially normal. A chest roentgenogram showed bilateral apical infiltrates, suggestive of active pulmonary tuberculosis (figure 1). The tuberculin skin test was negative to 1.0 mg. of OT. The histoplasmin, blastomycin, and coccidioidin skin tests were also negative. Because of a positive complement-fixation test for the detection of antibodies to *Histoplasma capsulatum* of a 1:4 titer using the whole yeast antigen, and a 1:2 titer using histoplasmin, a diagnosis of acute pulmonary histoplasmosis was considered. Negative complement-fixation tests were obtained using the whole yeast of *Blastomyces dermatitidis* and the mycelial filtrate of *Coccidioides immitis* as antigens. These and subsequent complement-fixing titers to the mycotic antigens are shown in table 1.

After a four-day hospitalization, the patient was discharged to his home for rest and limited activity. Close follow-up observation was continued at the Macon County Tuberculosis Sanatorium. Six daily sputum specimens obtained at the end of September, 1956, were negative by culture

[1] From the Section of Preventive Medicine, University of Chicago School of Medicine, Chicago 37, Illinois; and Macon County Tuberculosis Sanatorium, Decatur, Illinois.

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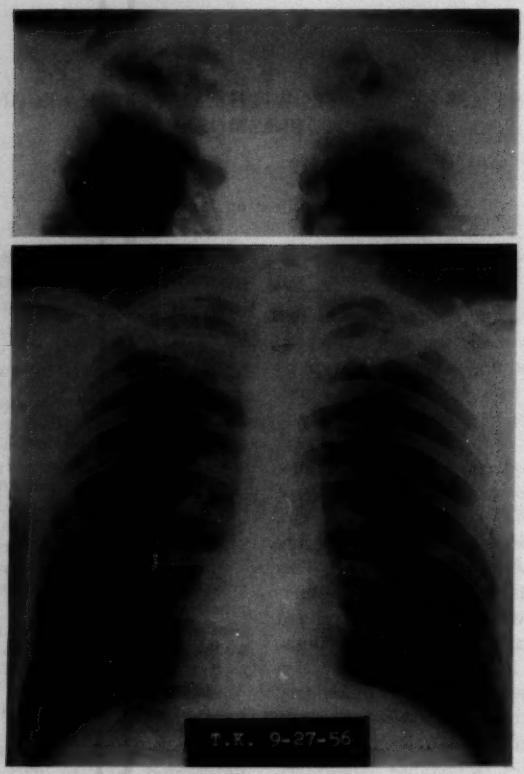


FIG. 1. Planigram and anteroposterior chest roentgenogram obtained on September 27, 1956, showing bilateral apical infiltrates due to acute histoplasmosis.

TABLE 1

COMPLEMENT-FIXATION TESTS FOR MYCOTIC INFECTION

Date	Antigens			
	H. Capsulatum Whole Yeast	Histoplasmin	*B. Dermatitidis* Whole Yeast	Coccidioidin
9–27–56	1:4	1:2	Negative	Negative
10–4–56	1:4	1:2	Negative	Negative
10–26–56	1:8	1:2	Negative	Negative
11–27–56	1:8	1:2	Negative	Negative
1–15–57	1:8	1:2	Negative	Negative
2–15–57	1:4	Negative	Negative	Negative
3–12–57	1:4	Negative	Negative	Negative
4–22–57	1:4	Negative	Negative	Negative
5–17–57	1:4	Negative	Negative	Negative
6–14–57	1:4	Negative	Negative	Negative
7–23–57	1:2	Negative	Negative	Negative
10–15–57	1:2	Negative	Negative	Negative
1–24–58	1:2	Negative	Negative	Negative
6–10–58	1:2	Negative	Negative	Negative
1–27–59	Negative	Negative	Negative	Negative
7–10–59	Negative	Negative	Negative	Negative
9–3–59	Negative	Negative	Negative	Negative

for tubercle bacilli and *H. capsulatum*. A Brucella agglutination test was negative. The diagnosis of acute pulmonary histoplasmosis was more emphatically substantiated on October 26, 1956, by the conversion of the histoplasmin skin test from a negative to a strongly positive reaction and the increase of the Histoplasma complement-fixation titer to 1:8.

Following discharge from the hospital, the patient continued to experience weakness, fatigability, exertional dyspnea, and intermittent cough productive of clear sputum. He was allowed out of bed only three to four hours a day through November, 1957. A three-day bout of "flu" with fever to 101°F. occurred in late December, 1956. The twenty-five-pound weight loss incurred during his acute illness was gradually regained and his weight was recorded at 156 pounds in mid-January, 1957. Monthly complement-fixation tests for Histoplasma antibodies remained at a 1:8 titer until January 15, 1957; for the next six months the titer was steady at 1:4. On March 12, 1957, the patient was essentially asymptomatic. He felt stronger and was out of bed ten hours or more a day. By June, 1957, he was able to tolerate twelve hours of farm labor daily. He weighed 161 pounds. The tuberculin skin test remained negative to 1.0 mg. of OT.

In October, 1957, the patient reported that he continued to feel very well. The Histoplasma complement-fixation titer had fallen to 1:2. Bimonthly chest roentgenograms had shown resolution and increasing fibrous scarring in the bilateral apical infiltrates. The right apex, in all probability, contained a thin-walled cavity (figure 2*A*, *B*). The impression, however, was that of inactive and healing histoplasmosis.

On January 24, 1958, roentgenograms of the stomach and colon, obtained because of gastrointestinal distress, were normal. The patient reported for the first time that he was occasionally raising blood-streaked sputum. Although his appetite was good, he had lost 5 pounds over a six month period. His only respiratory complaint wa

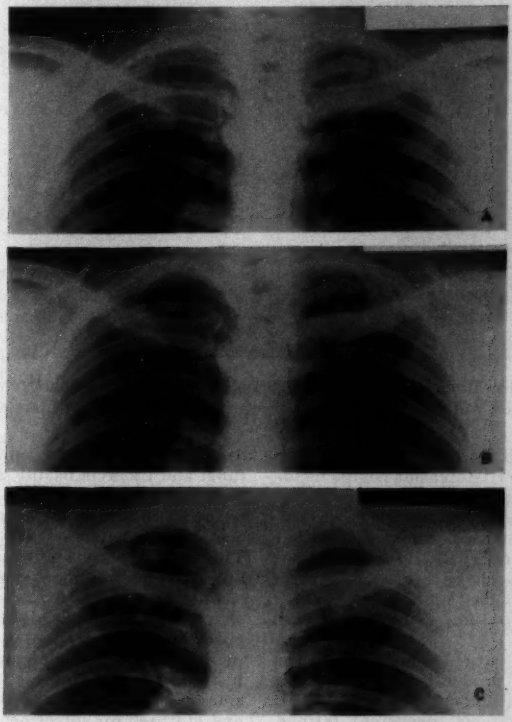


FIG. 2. Serial apical roentgenograms showing resolution of the acute bilateral Histoplasma infection, subsequently healing on the left by fibrosis and suggestive cavitation on the right. *A*. November 27, 1956—minimal clearing of both apical infiltrates. *B*. May 17, 1957—marked clearing and residual fibrosis of bilateral infiltrates. *C*. January 24, 1958—further clearing of right infiltrate with probable cavitation; increased fibrosis in left apex.

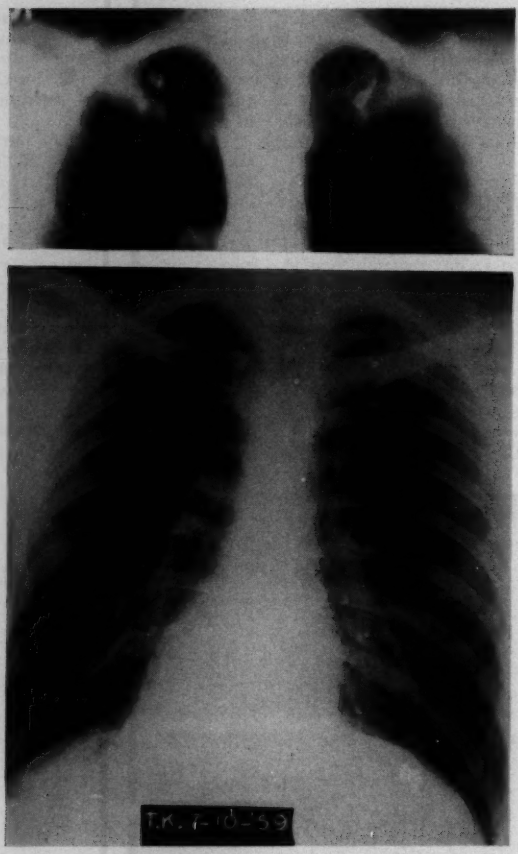


FIG. 3. Planigram in the 10-cm. plane and anteroposterior chest roentgenogram obtained on July 10, 1959, demonstrating thick-walled cavity in right apex and dense fibrosis in left apex.

dyspnea associated with increased abdominal distention. The titer of the Histoplasma complement-fixation test remained at 1:2 but fell to negative by January, 1959. The chest roentgenogram was unchanged except for increased fibrosis in the left apex (figure 2*C*). On January 27, 1959, the patient complained of a persisting severe cold of three weeks' duration. He coughed up large amounts of mucoid nonpurulent sputum, frequently tinged with blood.

By July 10, 1959, the patient reported more frequent coughing spells, productive of blood clots and blood-streaked sputum. Although he had frequent sore throats, dyspnea, persistent fatigue, and some frank left chest pain, there was no further weight loss. He complained of an attack of "flu" lasting three to four days, with low-grade fever, stomach upset, diarrhea, and headache. The Histoplasma complement-fixation test remained negative. Three sputum cultures were negative for fungi and tubercle bacilli. A chest roentgenogram demonstrated light fibrous infiltration in the extreme right apex with a thick-walled cavitation at the level of the fourth rib posteriorly (figure 3). The patch of fibrous infiltration in the extreme left apex had become more dense. Apical planigrams clearly defined a 1 by 2.5 cm. elongated peanut-shaped cavity in the right apex located slightly posteriorly and best seen in the 10-, 11-, and 12-cm. planes (figure 3). A diagnosis of chronic cavitary histoplasmosis with hemoptysis was strongly considered despite the fact that serologic reactions and cultures for infection with *Histoplasma capsulatum* were negative. Because of increasing sputum production, hemoptysis, and progressive debility, the patient was hospitalized at the University of Chicago Clinics for treatment.

Past history: The patient had had only the usual

childhood illnesses. He had a predisposition for frequent respiratory infections, characterized by tonsillitis at the age of seven, "strep throats" at seven and a half and twenty-six years, bronchitis at twelve, and frequent head colds and influenza-like attacks on four or five occasions during his lifetime. One such "flu-like" illness required hospitalization at Fort George, California, in 1951 while he served in the Armed Forces. Frequent postprandial indigestion and gaseous eructations, associated with occasional nausea, vomiting, constipation, and dull upper abdominal distress, had occurred in more recent years. Vasomotor instability, characterized by fainting, had been observed.

Social and family history: The patient had been actively engaged in farming for the past seven years and had lived most of his life on a farm near Illiopolis, Illinois, located close to Decatur. The chief crops on the farm were corn, soy beans, and wheat. Prior to his illness in 1956, he had raised chickens and cleaned the chicken coops weekly. During 1951 and 1952 he served with the Armed Forces at Fort George in southern California, except for three months spent in Austin, Texas. He had smoked one pack of cigarettes daily since 1951. There was no family history of tuberculosis nor were there any known tuberculous contacts.

In October, 1956, the patient's father and brother had had positive histoplasmin skin tests; the mother's skin test was negative. The father's chest roentgenogram showed evidence of healed granulomatous disease; the brother's roentgenogram was normal. Histoplasma complement-fixation titers on serum from the father and brother were negative and 1:2, respectively; that of the brother was negative one month later.

Physical examination: The patient was a tall, well-developed, well-nourished white male with ruddy complexion. He did not appear acutely or chronically ill. His weight was 154 pounds. The temperature, pulse, and respiratory rate were normal. The blood pressure was 126 systolic and 64 diastolic in mm. of mercury. A few small, nontender posterior cervical lymph nodes were present. The chest was clear to auscultation and percussion. A soft systolic murmur was heard over the entire precordium. There was no enlargement of the heart. The liver and spleen were not palpable. A small xanthoma was present on the palmar surface of the left ring finger.

Laboratory data: The leukocyte count was 10,200 per cu. mm. with 66 per cent polymorphonuclear leukocytes, 22 per cent small lymphocytes, 8 per cent monocytes, and 4 per cent eosinophils. The hemoglobin was 14.8 gm. per 100 ml., erythrocyte count 4.41 million per cu. mm., and hematocrit 42 per cent. The erythrocyte sedimentation rate was 6 mm. in one hour (Sahli method). The serologic test for syphilis (Kahn) was nonreactive. Urinalysis revealed a specific gravity of 1.022, absence of albumin or sugar, and a rare erythrocyte on microscopic examination. Tests of liver function were normal. A nonfasting blood glucose was 111 mg. per 100 ml. The blood urea nitrogen was 12 mg. per 100 ml.

Tests of pulmonary function demonstrated a vital capacity of 5.0 liters, inspiratory capacity of 3.1 liters, expiratory reserve of 1.9 liters, residual volume of 2.7 liters, functional residual capacity of 4.4 liters, and total lung capacity of 7.7 liters. The timed vital capacity was 75 per cent, expired in one second. The maximal breathing capacity was 137 liters per minute; diffusing capacity, 26 cc. per minute per ml. of mercury; and nitrogen washout index, 0.5 per cent. The oxygen saturation at rest was 97.5 per cent with air and 100 per cent with pure oxygen. These pulmonary function tests were considered entirely normal.

Skin tests to second-strength PPD, coccidioidin, and blastomycin remained negative. The histoplasmin skin test was positive. The complement-fixation tests for histoplasmosis, coccidioidomycosis, and blastomycosis were negative. Two sputum smears were negative for acid-fast bacilli. Seven additional sputum specimens, four of which were morning specimens and three twenty-four-hour collections, were cultured for fungi on Sabouraud agar, enriched blood agar, and corn meal agar. The twenty-four-hour sputum collections amounted to 10 to 15 ml. of clear mucoid material. Several specimens contained blood flecks. A few colonies of Aspergillus were cultured from two of the morning specimens but were considered contaminants. In particular, *Histoplasma capsulatum* could not be identified in the sputum by direct culture nor by the mouse-passage technique.

The admission chest roentgenogram showed bilateral apical fibrous infiltrates, more densely localized on the left, with cavitation on the right. An electrocardiogram was normal. Bronchoscopy was not performed.

Hospital course: Because of the presumptive diagnosis of cavitary histoplasmosis well localized to the right apex, resulting in increasing debilitation and hemoptysis, the patient was considered a suitable candidate for surgical excision of the lesion. A prophylactic course of intravenous amphotericin B[3] was given for six days preoperatively. The daily dosage was increased slowly from 35 to 50 mg. In spite of the concomitant use of sodium amytal, salicylates, and antihistaminics, the toxic manifestations of fever, chills, nausea, vomiting, and apprehension occurred.

On September 10, 1959, a segmental resection of the apical and posterior segments of the right upper lobe was performed without incident by Dr. William Adams. Extensive retraction of tissue was noted in the apex of the right upper lobe, involving both of these segments and an area of emphysematous lung adjacent to them. Scar tissue could be palpated deeply within the apex. There were no adhesions or inflammatory involvement of the parietal pleura or mediastinal tissues. Regional adenopathy was minimal, and a few small nodes in

[3] Kindly supplied under the trade name of Fungizone by E. R. Squibb and Company.

the superior portion of the hilum were removed for culture and pathologic examination. The surgical specimen appeared to be involved grossly with cavitary granulomatous disease.

Postoperatively, the patient was continued on 50 mg. of intravenous amphotericin B for three days. Fever spikes occurred until amphotericin B was discontinued. Thereafter, a low-grade fever persisted despite treatment with penicillin (600,000 units twice a day) and streptomycin (0.5 gm. twice a day). The blood urea nitrogen had elevated slightly to 23 mg. per 100 ml. by the day of surgery and quickly fell to normal after discontinuation of amphotericin B. Only 500 ml. of plasma and no whole blood were required during the surgery. The leukocyte count ranged from 10,450 to 21,900 per cu. mm. postoperatively.

Although the patient quickly regained his appetite and physical strength, he developed a right bronchopleural fistula and pneumothorax. In spite of 50 per cent glucose and streptokinase instillation and apical placement of a Bardex catheter for suction, the postoperative complication persisted.

On October 5, 1959, a right extrafascial thoracoplasty was performed. The Bardex catheter in the apical space was maintained on suction. One thousand milliliters of whole blood were administered during the surgery. The patient became afebrile by the fourth postoperative day. He was out of bed freely by October 9, 1959. Subsequent chest roentgenograms demonstrated disappearance of the pneumothorax and resolution of the pleural effusion. The patient was discharged on October 14, 1959.

When seen several weeks after discharge his weight was 151 pounds. He was gaining strength and offered no specific complaints. The chest was clear to percussion and auscultation below the wax pack. Fluoroscopic examination of the chest revealed a small amount of fluid in the right costophrenic angle and absence of parenchymal infiltrates. The diaphragms moved well. He was encouraged to begin activity on the farm.

Pathology: The surgical specimen consisted of the left apical and posterior segments, containing a solitary 1 by 1 cm. cavity in the apex. Grossly, the walls of the cavity were pale, firm, and irregular. Microscopically, the cavity wall was fairly thick and lined primarily with granulation tissue (figure 4). It was composed of granulomatous tissue and contained a few focal areas of necrosis and calcification. A marked chronic inflammatory response was present in the fibrotic capsule and consisted of large numbers of lymphocytes and plasma cells, some epithelioid cells, and only occasional giant cells. The abscess cavity possessed only small areas of epithelial lining demonstrating squamous metaplasia. The outer portions of the wall consisted of dense, somewhat hyalinized connective tissue.

The lumen of the cavity contained masses of interwoven fungal hyphae highly suggestive of Aspergillus (figure 5*A*). The hyphae were wide and septate with rather thick conspicuous walls (figure 5*B*). The mycelia were infiltrated with leukocytes, primarily polymorphonuclear in type. No fructification forms were present. The cavity obviously communicated with a bronchus which was surrounded by a chronic inflammatory reaction (figure 6). Masses of hyphal elements had filled the

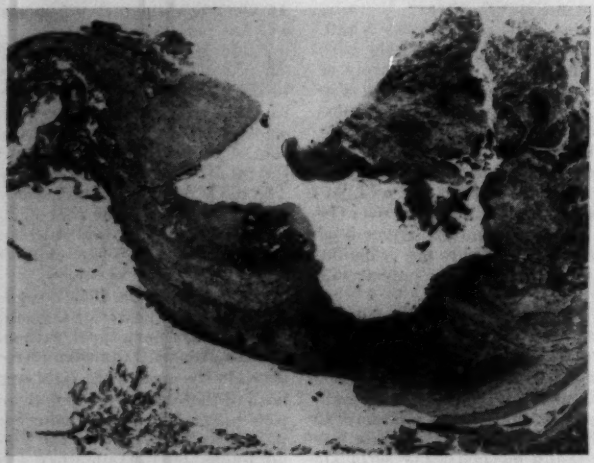
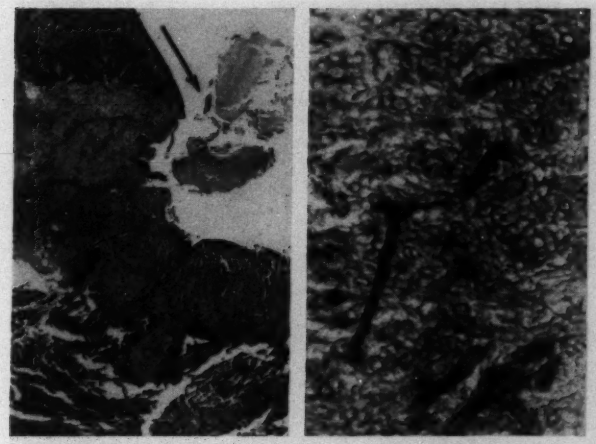


FIG. 4. Photomicrograph of a portion of the resected cavity. The wall is thickly fibrotic and markedly infiltrated with lymphocytes and plasma cells. A small area of squamous metaplasia can be seen just before the cavity communicates with the mycelial-plugged bronchus at the upper right (arrow). An area of calcification is present within the cavity wall. The area indicated by the arrow is further magnified in figure 6. (Hematoxylin and eosin stain, ×8)



A B

FIG. 5. *A*. Photomicrograph showing mycelial masses of Aspergillus occupying a portion of the apical cavity (arrow). Most of the cavitary content had been removed for culture. (Hematoxylin and eosin stain, ×17) *B*. Interwoven mass of septate hyphae of Aspergillus from cavity at site of arrow (*A*). (Gridley stain, ×500)

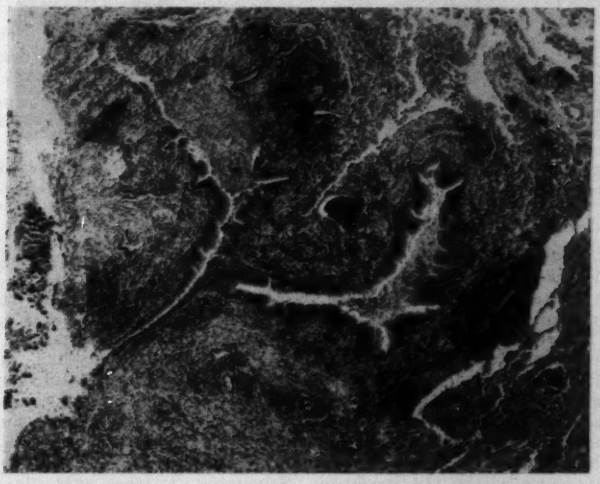


FIG. 6. Photomicrograph of area indicated by arrow in figure 4 showing bronchus, filled with hyphal elements, communicating with the cavity at the upper edge of picture. Portions of the epithelial lining have been destroyed, but the fungus remains confined to the bronchus. Intensive inflammatory reaction surrounds the bronchus. (Hematoxylin and eosin stain, ×40)

lumen of the bronchus but had not penetrated its lining. The epithelial lining itself had been partially destroyed. Fresh and old fibrosing thrombi were noted about the cavity wall.

The lung parenchyma surrounding the cavity wall was also chronically inflamed. Large focal lymphocytic infiltrates were grouped in areas where such chronic inflammation was not associated with the presence of Aspergillus organisms. A few typical well-organized old granulomas with caseous centers were located in such areas (figure 7). Further evidence of a more chronic granulomatous process than that due to Aspergillus infection was located within the posterior segment and was

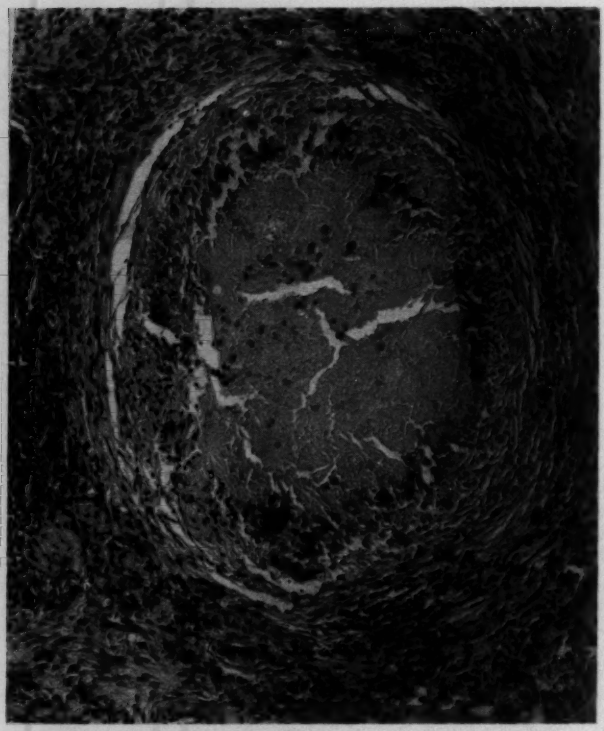


FIG. 7. Photomicrograph of a caseating granuloma typical for histoplasmosis within an atelectatic chronically inflamed area of the lung located peripherally to the cavity. (Hematoxylin and eosin stain, ×115)

completely separated at the interspace from the apical segment. Pleural scarring and retraction were present at the site.

At the resected margin of the bronchi, dilatation was present. The epithelium was somewhat eroded, but there was little evidence of inflammation. In uninvolved peripheral lung, both posterior and anterior sections showed some atelectasis but minimal inflammatory response. In certain areas of atelectasis, bronchi appeared to be totally collapsed, although the blood vessels remained patent. Several lymph nodes within the specimen showed marked reticulum cell hyperplasia and moderate anthracosis.

Numerous fungal stains, including the Gridley and Gomori stains, failed to demonstrate any fungi organisms within the wall. In particular, Histoplasma organisms or their chitinous ghosts could not be found within any of the macrophages of the granulomatous cavity wall, the cavitary content, or caseous centers of old granulomas. The intertwining mycelial masses were obvious within the cavity and communicating bronchus by these staining techniques. The impression was that of an old healed granulomatous infiltrate containing an active Aspergillus cavity.

Culture: Immediately following surgery, the discrete thick-walled cavity was opened aseptically for the purpose of culturing the contents. It contained a brown putty-like, crumbly material, which was packed snugly into the cavity and could be dislodged by a cotton swab only with some effort. Portions of the cavity content were swabbed directly onto mycologic media, and saline suspensions were prepared for mouse inoculation. Sabouraud dextrose agar, dextrose-cysteine-enriched blood agar, and corn-meal agar were used, and cultures were incubated at 20° and 37°C. Routine bacterial cultures and smears for acid-fast bacilli were also made from the material. Portions of the cavity wall, adjacent parenchyma, peripheral lung, and several lymph nodes were individually ground with sterile sand for direct culture and inoculation into mice. The smears and cultures for acid-fast organisms and other routine bacterial cultures were negative.

Within three days, all of the plates inoculated with the contents of the cavity and portions of

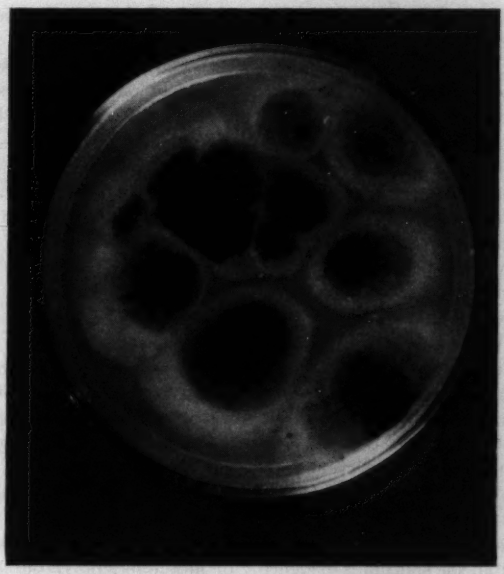


FIG. 8. Pure culture of *Aspergillus fumigatus* on Sabouraud agar incubated at room temperature for five days obtained from cavity.

cavity wall contained pure growths of fluffy white mycelia. By the end of a week, the colonies had overgrown the surface of the medium and turned increasingly green in color (figure 8). It is interesting to note that this fungal organism was at first considered a probable laboratory contaminant. However, with the overwhelming purity of culture from the cavity, proper respect was given to this fungus as the etiologic agent. Direct microscopic examination of the fungus revealed the typical septate hyphae, conidiophores, sterigmata, and chains of spores of *Aspergillus fumigatus*.

All of the other tissues from the surgical specimen showed no fungal growth other than a penicillium contaminant from the peripheral lung. Specifically, *Histoplasma capsulatum* was not isolated by direct culture from any samplings of the surgical specimen, although it might easily have been overwhelmed by the more rapidly growing Aspergillus organism. All cultures done from the reticuloendothelial organs of mice infected with sputum and surgically excised tissue were also negative for *Histoplasma capsulatum*.

DISCUSSION

The difficulty in differential diagnosis between cavitary aspergillosis and cavitary histoplasmosis demonstrated by this case report is punctuated by the comparative rarity of the former and the increasing awareness of the latter as a rather common form of the disease. It has been estimated, on the basis of serologic studies, that 10 per cent of patients in tuberculosis hospitals located within the Histoplasma endemic area have chronic pulmonary histoplasmosis (2). The chronic cavitary form of histoplasmosis has been reported with increasing frequency (3–5). Like aspergillosis, histoplasmosis has been frequently misdiagnosed as tuberculosis, since all three of these infections are clinically and roentgenographically indistinguishable. However, many tuberculosis hospitals can now pride themselves on the ability to differentiate between histoplasmosis and tuberculosis by cultural, serologic, and skin-testing techniques.

A presumptive diagnosis of acute bilateral apical histoplasmosis was logically warranted in this case during the fall of 1956. The patient had been a lifelong resident of southern Illinois where the prevalence of histoplasmin skin sensitivity exists in approximately 75 per cent of the population (6). The conversion from a negative to a positive histoplasmin skin test in the course of a clinically and roentgenographically apparent infection, while the tuberculin and other fungal skin tests remained negative, provided excellent

evidence for exposure to the Histoplasma organism. Since cultural methods failed to provide a definitive diagnosis, as they so often do in acute pulmonary histoplasmosis, the positive serologic test with a rise and later fall in titer provided additional indispensable proof of *Histoplasma capsulatum* as the etiologic agent and its subsequent eradication from the tissue. The long experience of the present writers with the complement-fixation test in the diagnosis and prognosis of histoplasmosis allows for such an interpretation only on the basis of serial determinations of titer, not on the degree of elevation of the titer.

Cavitation within the right apex probably occurred rather early during the resolution and fibrosis of the acute histoplasmosis. The cavity probably existed long before the patient's first episode of hemoptysis. Whether necrosis and cavitation were due entirely to reinfection with the Histoplasma organism is impossible to determine. The disappearance of complement-fixing antibodies to *H. capsulatum* and the inability to culture the organism from the sputum is evidence against the continuing presence of this organism within the lesion. However, because of the patient's endemic exposure, the presumptive evidence for acute histoplasmosis, and the fact that almost 10 per cent of patients with cavitary disease may have negative serologic tests (5), cavitary or reinfection histoplasmosis remained the preferred diagnosis.

However, it is more logical to assume that the Aspergillus fortuitously invaded a pre-existing focus of Histoplasma infection as a result of environmental exposure. The right apical infiltrate or cavitation served as the diseased tissue subjected to secondary invasion by the ubiquitous saprophyte, *Aspergillus fumigatus*. The secondary invader could have easily overwhelmed the primary agent. The actual diagnosis of aspergillosis had not been considered before surgery. The frequent occurrence of Aspergillus as contaminants of laboratory cultures causes great difficulty in making such a diagnosis. Even the repeated isolation of Aspergillus from sputum or other clinical materials in the absence of a more likely pathogen may still not be conclusive proof of the infection. The microscopic demonstration of the organism in histologic material together with cultural identification was necessary to establish properly the diagnosis of aspergillosis.

The treatment of choice for the patient's mycotic infection was identical, regardless of whether it was caused by the Aspergillus or Histoplasma organism. Surgical excision for localized lesions has provided significant numbers of successful cures in both cavitary aspergillosis and cavitary histoplasmosis (7, 8). The prognosis of either of these chronic mycotic pulmonary infections is poor if the patient is not treated.

Although drug therapy for these two mycotic infections has not been consistently effective, amphotericin B, alone or combined with surgical therapy (5, 7, 9, 10) has proved significantly valuable in the treatment of chronic histoplasmosis. There is reason to suppose that a prophylactic course of this antifungal agent before surgical removal of a Histoplasma cavitary lesion will sterilize the tissues and prevent risk of local contamination or dissemination with the organism.

Evidence has also been increasing recently to warrant further trials of amphotericin B in the treatment of aspergillosis. The inhibitory effect of amphotericin B on Aspergillus is not pronounced *in vitro* (11, 12). However, in *in vivo* studies on rabbits infected with Aspergillus spores, lesions were rare when the animals were treated with amphotericin B immediately after inoculation, although positive cultures were obtained at autopsy (12). Several clinical trials with this antimicrobial in the treatment of aspergillosis are encouraging. Newcomer and associates (13) treated two patients with pulmonary aspergillosis, one of whom showed partial healing of a draining sinus after six months of therapy. Closure of a bronchopleural fistula due to *Aspergillus fumigatus* in a tuberculous patient was effected temporarily by treatment with amphotericin B in another clinical trial (14).

Nystatin has also proved somewhat effective against aspergillosis. Ridell (15) found that brilliant green or hydroxystilbamidine solutions and nystatin in suspension were effective in freeing *Aspergillus fumigatus* from bronchial secretions. Two cases of Aspergillus infection of the pneumonectomy space following surgery for pulmonary carcinoma were successfully treated with instillation of aqueous suspensions of nystatin (100 and 500 units per ml.) by Sochocky (16).

Summary

The case of a patient with cavitary aspergillosis superimposed upon bilateral apical histoplasmosis

is presented. The difficulty in differential diagnosis between the cavitary forms of these two mycotic infections is discussed. Therapeutic measures for each infection are compared.

Sumario

Aspergilosis Pulmonar con Cavernas Secundarias a Histoplasmosis

Preséntase el caso de un enfermo con aspergilosis cavitaria sobrepuesta a histoplasmosis bilateral del vértice. Se discute la dificultad con que tropieza el diagnóstico diferencial entre las formas cavitarias de esas dos infecciones micóticas. Se comparan las providencias terapéuticas para cada infección.

Resume

Aspergillose pulmonaire avec caverne secondaire à une histoplasmose

L'auteur présente le cas d'un malade porteur d'une caverne due à une aspergillose à laquelle se superposa une histoplasmose bilatérale de l'apex. L'auteur discute la difficulté du diagnostic différentiel entre les formes cavitaires de ces deux infections mycotiques. Il compare les mesures thérapeutiques pour chaque infection.

Acknowledgments

The writers acknowledge the helpfulness of Dr. Eleanor M. Humphreys and Dr. F. Lamont Jennings, Department of Pathology, University of Chicago, in the interpretation of the tissue pathology; and the fine surgical skills of Dr. William Adams and his associates in the Department of Surgery.

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Editorial

The Fallacy of Diagnosing Tuberculosis without Proof of Etiology[1]

Soon after the epochal observations of Robert Koch establishing that tuberculosis is an infectious disease caused by a specific bacterium, controversy arose and has continued concerning the precise nature of the infective agents associated with tuberculosis in different species of animals. Despite the views of many distinguished scientists, Koch refused for many years to accept the idea that the Mycobacterium of bovine tuberculosis was capable of producing serious disease in man. Furthermore, in the face of impressive evidence to the contrary, Koch persisted in his belief that the tubercle bacillus was always the same regardless of the host it might inhabit naturally. In fact, as late as 1890, he held that all forms of tuberculosis were etiologically identical. Not until 1901, nineteen years after he announced the discovery of the tubercle bacillus, did Koch retreat from his previous position and state that, in his opinion, tuberculosis of poultry was quite dissimilar to tuberculosis of human beings and tuberculosis of human beings was unlike that of cattle. Thus, one controversy was finally terminated.

In retrospect, Koch's debate with his many critics exemplifies the value of controversy when the issues are examined objectively. Such controversies usually result in accelerated efforts to obtain more information. Eventually facts, rather than opinions, provide a rational resolution of the dispute.

The preceding remarks are a prelude to the consideration of a contemporary question of much significance to all workers concerned with tuberculosis and other mycobacterial infections. The question has two parts and may be stated briefly as follows:

I. How may one define the disease tuberculosis?

II. Should granulomatous pulmonary lesions containing only mycobacteria such as are listed in the classification of Runyon (Runyon, E. H.; Med Clin N Amer, 1959, *43*, 273) be designated tuberculous even though tubercle bacilli of human, bovine, or avian origin are not demonstrable?

We are all familiar with the standard medical dictionary definition of tuberculosis as "an infectious disease caused by *Myco. tuberculosis*, and characterized by the formation of tubercles." It appears that some observers would prefer a less circumscribed definition, i.e., one that is less precise, particularly with regard to the character of the etiologic agent. These workers might define tuberculosis as a nonspecific mycobacterial infectious disease of variable, inconstant, or unknown etiology, and characterized by a morbid state, the important features of which are tubercles, necrobiosis, and giant cells of the Langhans' type. In this concept of the disease, the character of the tissue reaction of the host to the bacterial invader, rather than the exact character of the infective agent, is accepted as pathognomonic.

As a matter of fact, in man, tubercles and giant cells of the Langhans' type occur in a long list of granulomatous conditions other than the disease caused by the tubercle bacillus. A partial list, showing the diversity of morbid conditions in which tubercles may be present, includes such specific entities as lymphogranuloma inguinale, tularemia, brucellosis, tuberculoid leprosy, syphilis, typhoid fever, infection with *Schistosoma mansonia*, so-called "skin tuberculosis" of cattle, sarcoidosis, berylliosis, the intracutaneous tuberculin reaction, silicosis, and certain fungous infections such as nocardiosis. An impressive example of a granulomatous pulmonary lesion previously considered, without etiologic proof, to be tuberculosis is the so-called "tuberculoma." More recently it has been shown repeatedly that, when such lesions are examined microscopically, following appropriate staining, Histoplasma or other fungi are found to be present in a high percentage of the cases. (Zimmerman, L. E.: Trans NY Acad Sci (Ser II) 1957, *19*, 358).

The previously mentioned array of nontuberculous conditions serves to deprive the tubercle of much of its significance as an indicator of any specific morbid entity. The same is true for the Langhans' type of giant cell in the detection of tuberculous infections.

It is obvious, at least to me, that certain morphologic features associated with many granulomatous lesions, including tuberculosis, are nonspecific increments of the tissue response and

[1] Presented at the Nineteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces, 1960.

are definitely unreliable criteria on which to base a precise diagnosis.

On the basis of available information, I believe that it is unfortunate and unwise to designate clinical conditions as tuberculosis when mycobacteria definitely distinguishable from tubercle bacilli are present, but proved tubercle bacilli are not demonstrated. To insist on designating such infections as tuberculosis is to rely on an untenable presumption rather than on facts. Furthermore, the acceptance of the idea that pulmonary tuberculosis of man may be caused by species of mycobacteria other than *M. tuberculosis* would seriously retard the future development of our knowledge of the pathogenesis of pulmonary tuberculosis. This would be regrettable.

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Notes

THE INCIDENCE OF HYPERSENSITIVITY TO MAMMALIAN AND BATTEY PPD IN MEDICAL AND DENTAL STUDENTS

Interest in the specificity of the tuberculin test in human beings has been reawakened by the results of numerous studies conducted in recent years by Palmer and associates.[1-8] Their accumulated evidence has led these investigators to the conclusion that naturally acquired sensitivity to mammalian tuberculin is not exclusively the result of infection with typical mammalian tubercle bacilli. They believe that infection with *M. tuberculosis* or *M. bovis* can almost always be revealed by a definite reaction with a small (5 TU) dose of tuberculin made from mammalian mycobacteria. As these reactions supposedly indicate sensitivity produced by actual infection, they are referred to as specific, whereas reactions which occur only when much larger doses of mammalian tuberculin are administered are called nonspecific. They are supposed to indicate sensitivity produced by infection produced by an acid-fast organism different from, but antigenically related to, the organism from which the tuberculin was made.

The cause of this so-called nonspecific sensitivity to mammalian tuberculin among human beings has remained unknown, but in 1958 Edwards and Palmer[9] reported that PPD prepared from a strain of Battey unclassified acid-fast mycobacteria produced much larger dermal reactions in patients having disease produced by a Battey microorganism than in patients suffering from disease caused by *M. tuberculosis*. On the other hand, patients with disease caused by *M. tuberculosis* gave much larger dermal reactions following injection with regular mammalian tuberculin than did patients suffering from disease produced by the Battey microorganism.

In the same study, Edwards and Palmer skin-tested a large number of Navy recruits with 0.0001 mg. of both Battey and regular PPD. They found that the frequency of reactions to Battey PPD ranged from about 30 per cent in recruits from the northeastern part of the United States to about 65 per cent in those from the southeastern part, whereas reactions to regular PPD occurred only in 6 to 9 per cent of the recruits.

These results of Edwards and Palmer provided direct support for their hypothesis that a large proportion of those people who show a low degree of hypersensitivity to tuberculin prepared from mammalian tubercle bacilli actually may have been infected with a related but different Mycobacterium, and, furthermore, a Mycobacterium with a much lower capacity to produce clinical disease in human beings than *M. tuberculosis*. More recently Edwards and Palmer[10] have reported that unclassified mycobacteria, including the Battey type of microorganism, were found in the sputum or saliva of a surprisingly high proportion of the healthy adult population of a small rural community in Georgia.

At Northwestern University Medical School the incidence of reactors to second-strength regular PPD (0.005 mg.) among medical and dental students has remained high (40–60 per cent), although the occurrence of tuberculous disease in this population has almost disappeared.

In the fall of 1958, Dr. Lydia Edwards and Dr. Carroll Palmer kindly supplied this laboratory with purified mammalian tuberculin (PPD-S) and purified tuberculin prepared from the unclassified Battey microorganism (PPD-B).

The members of the sophomore medical and dental classes and a few junior and senior dental students were tested by injecting 0.0001 mg. (5 TU) of each preparation contained in 0.1 ml. of diluent[11] into the volar surface of the forearm. The

[1] Edwards, L. B., *et al.*: Pub Health Rep, 1958, *63*, 1569.

[2] Goddard, J. C., *et al.*: Pub Health Rep, 1949, *64*, 820.

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[8] Edwards, L. B., and Krohn, E. F.: Amer J Hyg, 1957, *66*, 253.

[9] Edwards, L. B., and Palmer, C. E.: Amer J Hyg, 1958, *68*, 213.

[10] Edwards, L. B., and Palmer, C. E.: Amer Rev Resp Dis, 1959, *80*, 747.

[11] Received dissolved, diluted, and ready to inject from Dr. Lydia Edwards, Public Health Service, Washington, D. C.

TABLE 1

NUMBER OF DERMAL REACTIONS GREATER THAN 6.0 MM. IN DIAMETER FOUND IN MEDICAL AND DENTAL STUDENTS GIVEN PPD-S AND PPD-B

	Medical Students		Dental Students		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Students tested with 5 TU PPD-S and PPD-B	110	100.0	68	100.0	178	100.0
Positive to 5 TU PPD-S	22	20.0	5	7.3	27	15.2
Positive to 5 TU PPD-B	32	29.1	16	23.5	48	27.0
Positive to 5 TU PPD-S and 5 TU PPD-B	17	15.4	4	5.9	21	11.8
Positive to 5 TU PPD-S only	5	4.5	1	1.5	6	3.4
Positive to 5 TU PPD-B only	15	13.6	12	17.6	27	15.2
Students tested with 100 TU PPD-S	63	57.3	40	58.8	103	57.8
Positive to 100 TU PPD-S only	43	38.2	19	27.9	62	34.8

PPD-S was injected into the skin of one arm and the PPD-B was injected into the skin of the other. The test sites were examined forty-eight hours later and the diameter of the indurated area, if present, was measured by one of us (G.P.Y.) without having any knowledge of which preparation had been injected into which arm. In order to avoid recognition of some pattern in the occurrence of the reactions, the persons who administered the tuberculins (R.P. and A.S.Y.) changed randomly the injection of the preparations from arm to arm. The students found to give no reaction to PPD-S, including the ones who reacted only to PPD-B, were retested by injecting 0.002 mg. (100 TU) PPD-S.

The results obtained following skin testing with the 0.0001 mg. doses of PPD-S and PPD-B are summarized in table 1.

The fairly small number of reactors to PPD-S is in line with general experience in these population groups, although the incidence is appreciably higher in the medical than in the dental students. What is of primary interest and in keeping with the findings of Edwards and Palmer[9] among Navy recruits is the larger number of students who gave reactions to PPD-B. Although not shown in table 1, calculation reveals that 40.5 per cent of the 37 medical students and 70.6 per cent of 17 dental students who gave reactions either to PPD-B or to first-strength PPD-S reacted only to the Battey PPD. Therefore, whether viewed from the standpoint of the number of reactors of the total number of students tested or from the standpoint of the proportion of those students who gave reactions, the incidence of hypersensitivity to PPD-B was much greater than to PPD-S.

Seventeen medical students and 4 dental students reacted to 5 TU of both PPD-S and PPD-B. Of this group, 3 of the medical students and 2 of the dental students had reactions to PPD-B which were very much larger than to PPD-S. These could very well represent a nonspecific Battey type of infection. The size of the two reactions noted in the remaining 16 students, however, did not differ by more than 5 mm. and many were of equal size. Such responses might indicate previous infection with both typical and unclassified mycobacteria. The average diameters of all of the reactions to PPD-S in this group was 13.8 mm.; to PPD-B, 20.0 mm.

The 15 medical students and 12 dental students who reacted initially only to PPD-B also were retested, using 0.002 mg. (100 TU) of PPD-S. Fourteen (93.3 per cent) of the former and 10 (83.3 per cent) of the latter showed reactions forty-eight hours later. The average diameter of the reactions in this group elicited by PPD-S was 10.5 mm.; by PPD-B, 16.1 mm.

Sixty-three medical students and 40 dental students who gave no reactions with either 5 TU PPD-S or 5 TU PPD-B also were retested, using 100 TU of PPD-S. Forty-three (38.2 per cent) and 19 (27.9 per cent), respectively, gave indurated reactions measuring more than 6 mm. Whether these reactions represent specific or nonspecific infection cannot be ascertained from these data. Unfortunately, PPD-B of an equivalent strength was not available, so comparison of the size of the reactions to 100 TU doses of the tuberculins could not be made. However, in view of the high incidence of nonspecific infection revealed by the tuberculins of weaker strength, it is reasonable to assume that a high proportion of the students who showed skin reactivity only to the high concentration of PPD-S actually had been infected with a nonspecific Mycobacterium. However, one should not assume that all low degrees of tuberculin hypersensitivity are nonspecifically engendered. There is no biologic law which requires that each person infected with *M. tuberculosis* develop such a degree of hypersensitivity that he will react to the small (5 TU) dose of tuberculin.

The majority of Northwestern University medi-

cal and dental students come from the middle western states and most of the remainder from the western states. Only 7 of the students tested in this survey came from the Southeast, where Edwards and Palmer noted the highest incidence of sensitivity to Battey PPD. Three of these 7 gave reactions following injection of PPD-B.

Edwards and Palmer[9] found that 30.0 per cent of Navy recruits from the northeastern section of the United States reacted positively with PPD-B. In a more recent publication, Edwards, Edwards, and Palmer[12] found that navy recruits from the Middle West and West also had a high incidence of reactions to PPD-B. The finding that 27.0 per cent of the students reacted to 5 TU PPD-B is in accord with that of Edwards, Edwards, and Palmer.[12]

[12] EDWARDS, L. B., *et al.*: Acta Tuberc Scand, 1959, Supplement 47, p. 77.

In summary: One hundred and seventy-eight medical and dental students at Northwestern University were skin-tested with 5 TU of PPD prepared from the unclassified Battey Mycobacterium and with 5 TU of PPD prepared from *M. tuberculosis*. Fifty-four students (30.3 per cent) gave reactions; 21 (11.8 per cent) reacted to both tuberculins, 27 (15.2 per cent) reacted only to PPD-B, and 6 (3.4 per cent) only to PPD-S.

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BRONCHOSCOPY MADE EASY

To the Editor of the American Review of Respiratory Diseases:

Physicians who count the ability to bronchoscope among their professional skills remember the trying times when, while a novice of the art, they stumbled around from vallecula to pyriform sinus until—oh happy day—the baffling brass pipe was finally and triumphantly inserted into the esophagus. As this groping proceeded, the instructor assisted by trying to push the larynx into the field of vision of the bronchoscope, suggested that one was to the right, left, anterior, or posterior of the bull's-eye and, finally, when the physical and psychic endurance of patient, instructor, and sweating tyro was fast approaching the breaking point, would take over the procedure and rapidly insert the bronchoscope through the previously elusive laryngeal opening. Many of us, no doubt, carry scars to the ego from these encounters. Eventually, one would pass through the period when it was possible to enter the trachea with a fair degree of facility in the average case, but one would periodically fail and would resort to such measures as exposing the larynx with a laryngoscope and inserting the bronchoscope under direct vision, or scheduling the patient at a later date with more preoperative medication. Only with a great deal of practice is the point reached when one feels confident of initiating a bronchoscopic examination successfully every time.

Having been in the position of the neophyte and, what is probably more trying on the nerves, the instructor, the writer has given much thought to a method of simplifying this most difficult of endoscopic procedures. The method which has developed is simplicity itself, takes no special equipment or knowledge of anatomic landmarks in the oropharynx or laryngopharynx, and results in successful endoscopy almost without fail on the first attempt, unless the patient is most uncooperative.

The patient is placed on nothing by mouth for a minimum of six hours prior to the elective procedure. He is premedicated with a short-acting barbiturate, meperidine (Demerol®), and scopolamine, the amount being varied to correspond to the patient's weight and age. The pharynx is lightly anesthetized by a spray of 2 per cent tetracaine (Pontocaine®) solution introduced transnasally and transorally. The tracheobronchial tree is lightly anesthetized with 0.005 per cent tetracaine solution injected transorally through the glottis, employing a curved malleable silver cannula which holds the epiglottis forward. The patient is asked to take a big breath and, as he does, 2 or 3 ml. of the agent are injected by syringe through the cannula into the trachea. The cough this produces disseminates the anesthetic agent throughout the tracheobronchial tree.

A number 14 or 16F rubber catheter is then introduced transnasally into the posterior oropharynx and, employing the Haight maneuver,[1] the catheter is inserted blindly into the trachea as the patient takes a deep breath. This stimulates severe coughing which is controlled by successive injections of 0.5 per cent tetracaine solution through the catheter until the point is reached when injection of a portion of a milliliter of the solution fails to elicit cough, or elicits only a negligible cough.

A knot is placed in the end of the catheter in order to avoid the possibility of its being aspirated into the trachea. With the help of an assistant, the patient is positioned after the method of Jackson[2] and the bronchoscope is inserted into the trachea by following the catheter, which is maintained in a position posterior to the orifice of the bronchoscope. When the bronchoscope has entered the trachea, the catheter is withdrawn and the evaluation carried out.

One might raise the objection that spoon-feeding the embryo bronchoscopist in this manner is really impeding his reaching a true level of professional performance, since he does not become acquainted with the anatomic features of the laryngopharynx. This stand is valid in a limited sense, but there are simpler and less traumatic procedures available for evaluating this portion of the anatomy, and its interest to the bronchoscopist is nearly always only incidental. Certainly, the trauma to the patient is markedly reduced. This is a factor of importance in an institution treating tuberculosis, where advertising, often of an unfavorable kind, is by word of mouth between patients.

Anesthetizing the tracheobronchial tree by injection through a previously passed tracheal catheter is considered to be superior to other methods. The catheter may be inserted with minimal anesthesia to the pharynx. The amount

[1] HAIGHT, C.: Ann Surg, 1938, *107*, 218.

[2] JACKSON, C., AND JACKSON, C. L.: Bronchoesophagology, ed. 1, W. B. Saunders Company, Philadelphia, 1950, p. 40 (figure 30).

FIG. 1. This position, as mentioned by Haight,[1] with the trunk flexed forward, chin raised, and tongue out, facilitates passage of a transnasal catheter into the trachea.

injected through the catheter can be titrated by the patient's coughing to the point at which the cough reflex is just abolished. In this manner, there is less danger of untoward reactions from anesthetic overdosage. Since the trip of the bronchoscope through the pharynx into the trachea is direct and rapid, only minimal anesthesia is required in this area. All who have seen convulsions or death from cocaine or its derivatives or synthetic counterparts can appreciate the importance of being able to limit the amount of anesthesia to that which is just needed and no more.

The physician in an isolated area who occasionally-to-rarely finds it necessary to perform bronchoscopy, often as an emergency, might find this maneuver worth developing in order to be able to avoid the use of a laryngoscope for visualization prior to inserting the bronchoscope through the glottis.

This procedure is so simple that it quite possibly has been described in the past and may be in use elsewhere. However, I have never seen the method used in other clinics, have not found a description of this technique in the literature and, in discussing this matter with other bronchoscopists, have encountered no one who has used this method. Perhaps, like the wheel, it was so obvious it was overlooked. At the least, it has not received adequate publicity

In summary, a simple, easily taught method of anesthetizing the tracheobronchial tree and performing bronchoscopic insertion is described. This method is of particular value for the occasional bronchoscopist and the student.

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February 10, 1960

Books

CARDIOVASCULAR REHABILITATION. By Paul Dudley White, Howard A. Rusk, Bryan Williams, and Philip R. Lee. Pp. 155, McGraw-Hill Book Company, Inc., New York, 1957. Price, $6.50.

REHABILITATION OF THE CARDIOVASCULAR PATIENT. By Paul Dudley White, Howard A. Rusk, Philip R. Lee, and Bryan Williams. Pp. 176, McGraw-Hill Book Company, Inc., New York, 1958. Price, $7.00.

Rehabilitation in the area of cardiovascular disease, if not a concept new to this decade, has undoubtedly gained wider currency and been subjected to more systematic study during this period. A symposium held at the New York University—Bellevue Medical Center in 1956 (reported on in *Cardiovascular Rehabilitation*) brought together a group of cardiologists, specialists in industrial hygiene, specialists in rehabilitation, persons concerned with the community functions of the American Heart Association, and others. Emotional problems and physical problems of the patient with cardiovascular disease were discussed in reference to their influence on the effective functioning of the heart in gainful employment. A large portion of the discussion was divided logically under the three headings: practice, teaching, and research in cardiovascular rehabilitation.

Appearing in 1958 under the names of the same four who edited the proceedings of the symposium, the monograph on cardiovascular rehabilitation (*Rehabilitation of the Cardiovascular Patient*) is a systematic treatment of the subject. Rehabilitation in cerebrovascular disease is given more space than any other single topic, and legitimately so, since measures of physical therapy are most applicable to patients suffering from this form of cardiovascular disease. In the case of coronary artery disease, rheumatic heart disease, hypertension, and other illnesses, discussion becomes more diffuse. The scope can be indicated, however, by the authors' definition of rehabilitation: "all those services which the individual with a cardiovascular disability needs in order to live as rich, satisfying, and productive a life as possible."

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BRONCHOLOGIE. TECHNIQUE ENDOSCOPIQUE ET PATHOLOGIE TRACHEO-BRONCHIQUE. Vol. I–II. By André Soulas and Pierre Mounier-Kuhn. Pp. 1146, Masson et Cie, Paris, 1956.

This is a completely revised and enlarged edition of the book first published in 1949. The artistic solidity of these two handsome volumes tactfully predicts that they will be handled by a multitude of doctors for many years to come and, at the same time, tastefully advertises that the book-producing industry of France is second to none.

In the foreword, Dr. Robert Monod states his opinion that a new medical speciality has been born, and one is convinced after reading this book that his statement is not an exaggeration. The book begins with the history of bronchology, giving credit to all whose contributions helped the development of this branch of medicine. Well deserved tribute is paid to the great pioneer of bronchology, the late Dr. Chevalier Jackson, whom Dr. Soulas calls, with true French enthusiasm for everything superb, "the apostle of bronchology." Honorable mention is also given the "Mecca" of bronchology—Philadelphia, the city that contributed greatly to this new specialty. The ample list of references is a "Who's Who" in bronchology, manifesting the principle: *"la science n'a pas de patrie"* (science is universal).

In the first chapters of the book, the anatomy and physiology of the bronchi are presented. A true picture can be obtained of the bronchial tree after comparing the roentgenographic and bronchographic data with the topographic data, and then one is ready to undertake some further exploration by way of endoscopy. To do this, one must be familiar with the instruments, the technique of endoscopy, and the incidents and accidents one may encounter in bronchology. These are all found in the book.

The book then proceeds to the various bronchial disorders. Entire chapters deal with the bronchial syndrome, the different types of tracheobronchitis and bronchiectasis, presenting in logical order the history, etiology, pathogenesis, semeiology, differential diagnosis, prognosis, and treatment of each of the bronchial diseases. With the advent of antimicrobials, the chapter on syphilitic tracheobronchitis—dealing chiefly with tertiary syphilis—is only of historical significance.

The book presents a little extra joy to the phthisiologist in the form of what is almost a monograph describing bronchial tuberculosis, divided into three sections: bronchial involvement in primary tuberculosis, bronchial tuberculosis in postprimary disease, and solitary tuberculous bronchitis. The reviewer would like to express his hope that this book will attain a status of such authority that its definition and classification of bronchial tuberculosis and the interpretation of bronchoscopic findings in that disease will be accepted. It would help to make for uniform diagnosis by the endoscopic method, which territory at present is a real jungle—the various publications report bronchial involvement ranging from 3 per cent to almost 100 per cent.

The longest chapter, a large part of the second volume, is devoted to tumors of the bronchial tree. This chapter leaves the reader with the feeling that he might wish to know more. The basic information is there, but many questions are left unanswered due to the flaws in our present knowledge of oncology.

The next chapter on foreign bodies in the bronchi is quite technical, and never perfunctory. The last chapters deal with the traumas, deviations, compressions, malformations, and anomalies, with an extra chapter each on aerosols, pulmonary function studies, and bronchospirometry. The reviewer believes that the latter two chapters would be better placed in the first part part of the book—the role of photocinematography and television in bronchology.

Reading this book was a pleasant experience. A wealth of knowledge is offered by two brilliant medical scientists in the spirit of Schopenhauer's principle "authors should use common words to say uncommon things." The style of the book is clear and easy, free of scientific bombast, yet never pedestrian. A great value would be gained by its translation into English. It would not be fair to omit appraisal of the illustrations, which include many color plates. They are exceptionally good and in style with the general appearance of the book. In writing the preface, Chevalier Jackson judged this book indispensable for the world of medicine. I humbly agree with him.

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BOOKS RECEIVED

Significant Trends in Medical Research. Ciba Foundation Tenth Anniversary Symposium. Edited by G. E. W. Wolstenholme, Cecilia M. O'Connor, and Maeve O'Connor. Pp. 356, Little, Brown and Company, Boston, 1959. Price, $9.50.

The Lifespan of Animals. Volume 5, Ciba Foundation Colloquia On Ageing. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. 324, Little, Brown and Company, Boston, 1959. Price, $9.50.

Biochemistry of Human Genetics. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 347, Little, Brown and Company, Boston, 1959. Price, $9.50.

Cancer of the Cervix. Diagnosis of Early Forms. Ciba Foundation Study Group No. 3. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. 114, Little, Brown and Company, Boston, 1959. Price, $2.50.

Tagungsbericht zum 9. Kongreb der Süddeutschen Tuberkulosegesellschaft in Passau (21–24 Mai 1959). Edited by Friedrich Kreuser. Pp. 204, Georg Thieme Verlag, Stuttgart (Intercontinental Medical Book Corporation, New York, New York), 1960. Price, $3.20.

Anatomy. A Regional Study of Human Structure. By Ernest Gardner, Donald J. Gray, and Ronan O'Rahilly. Pp. 999, W. B. Saunders Company, Philadelphia and London, 1960. Price, $15.00.

The Health of Business Executives. The Transactions of a One-Day Conference Held in the Royal Festival Hall, London, 20th November 1959. Pp. 126, Chest and Heart Association, London, 1960. Price, 15s. or $3.25.

Diagnosis and Treatment of Diseases of the Trachea and Bronchi. By Herman J. Moersch and Howard A. Andersen. Pp. 108, Charles C Thomas, Springfield, Illinois, 1960. Price, $4.25.

Work and the Heart. Transactions of the First Wisconsin Conference on Work and the Heart. Edited by Francis F. Rosenbaum and Elston L. Belknap. Pp. 537, Paul B. Hoeber, Inc., New York, 1959. Price, $12.00.

Hypertensive Disease. Diagnosis and Treatment. By Sibley W. Hoobler. Pp. 353, Paul B. Hoeber, Inc., New York, 1959. Price, $7.50.

Encyclopedia of Medical Syndromes. By Robert H. Durham. Pp. 628, Paul B. Hoeber, Inc., New York, 1960. Price, $13.50.

Infectious Diseases of Children. By Saul Krugman and Robert Ward. Pp. 340, C. V. Mosby Company, St. Louis, Missouri, 1958. Price, $10.00.

Health Protection in the Rumanian People's Republic. A pictorial presentation by the Ministry of Health and Social Welfare, Bucharest, 1959.

American Thoracic Society

Medical Section of the National Tuberculosis Association

CHANGE OF NAME OF SOCIETY

On Tuesday, May 17, 1960, at the Hotel Biltmore in Los Angeles, by a standing majority vote of the membership present, the American Trudeau Society became the American Thoracic Society, thus culminating several years of interesting discussion and occasional controversy.

On two previous occasions the question of the appropriateness of the name Trudeau, in the light of the rapidly changing character of the program of the Society, had been brought to the attention of the membership, and each time the vote had been to retain the name of the distinguished physician and educator, Dr. Edward Livingston Trudeau.

Toward the end of the year of his Presidency of the Society (1958–1959), Dr. Daniel E. Jenkins appointed an *ad hoc* committee to "consider and recommend a plan of action to be followed relative to a change in name of the American Trudeau Society." This committee met in Chicago on May 23, 1959, and made recommendations to the Council of the Society which were adopted by the Council on the following day, May 24, 1959. These recommendations in effect provided for:

(*1*) The appointment of a new committee to select "a specific name which will be most appropriately descriptive of the activities of the Society."

(*2*) Councilors of the Society will be asked to accept the responsibility of explaining to the membership of the Society the background and reasons for the recommended new name.

(*3*) After an appropriate interval for the Councilors to have this opportunity, the proposed new name will be submitted directly to the membership of the Society by mail ballot.

President Mitchell immediately appointed a new committee, which met in Chicago on May 27, 1959, and unanimously recommended, in response to its charge, that the following name be submitted to the membership of the Society for its approval: THE AMERICAN THORACIC SOCIETY.

This committee supported its recommendation with the following points:

(*1*) Professional societies which are national in scope should have in their titles words which are descriptive of their activities rather than the name of a great man.

(*2*) The recommended name for the Society is believed to be that which is most descriptive of the Society's activities, both present and future.

(*3*) More than other possible designations considered by the committee, it best describes the professional fields of interest of the members of the Society.

(*4*) The proposed name clearly retains tuberculosis, and embraces other related fields and other respiratory diseases.

The committee also unanimously recommended that "any new name for the American Trudeau Society need not necessarily be reflected in the name of a state or other Trudeau society, and that such societies should be entirely free to make the determination as to their names within the framework of the organization."

On July 15, 1959, President Mitchell addressed a circular letter to the Representative Councilors on this subject, in accordance with the vote of the Council on May 14. Early in the fall of 1959, ballots were mailed to the approximately 5,000 members of the Society, requesting that they indicate their preference for or rejection of the proposed name, the American Thoracic Society.

Approximately 3,200 ballots were returned. These represent over 60 per cent of the Society's membership, but about 85 per cent of the members residing in the United States. The proposed new name was endorsed by 77 per cent of those returning the ballot.

Bylaws of the Society require that a change in name be submitted to the membership at their annual meeting, after appropriate circulation of the proposed amendment thirty days in advance. Following these procedures, as required in the Bylaws, the proposal was placed before the membership. And so the name, the American Trudeau Society, has passed into history, and the Society marches into the 1960's under its newly descriptive name, the American Thoracic Society.

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

NATIONAL TUBERCULOSIS ASSOCIATION FELLOWSHIPS

As the Medical Section of the National Tuberculosis Association, the American Thoracic Society (formerly the American Trudeau Society) provides fellowships and traineeships for the graduate education of investigators and teachers in the field of respiratory diseases and tuberculosis. The following types are offered:

Edward Livingston Trudeau Fellowships: A few fellowships at a higher level of training and award are offered to specially qualified candidates with an M.D. degree who have been assured of a continued teaching or research appointment upon completion of training. Trudeau Fellowships are awarded for one year, but may be renewed up to a total period of four years.

Research Fellowships: Postdoctoral fellowships are offered to candidates holding the degree of M.D., Ph.D., or Sc.D. for further training as scientific investigators.

Predoctoral fellowships are offered to graduate students with a bachelor's degree to work on a research project for an advanced degree other than M.D.

Teaching Traineeships: Physicians entering their second or third year of residency in Internal Medicine or Thoracic Surgery at a medical center who may be interested in a career of medical teaching are offered traineeships toward that end. Supplementation of these awards by the institution involved is not necessary.

Each applicant for an NTA fellowship or traineeship must have been accepted by the head of the department under whom he expects to work for the next academic year in this country. Awards are determined by individual circumstances and are paid directly to the Fellow on a quarterly basis. They are granted for one year, beginning on any date after April first, and are renewable for a total of three years.

All applications must be submitted by November 1st. Further information about Fellowships may be obtained upon request from:

Director of Medical Education
American Thoracic Society
1790 Broadway
New York 19, New York

COURSE IN LABORATORY METHODS IN THE DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Microbiology Laboratories of the Communicable Disease Center, Atlanta, Georgia, will offer a course in Laboratory Methods in the Diagnosis of Tuberculosis on the following dates:

October 31–November 11, 1960
January 30–February 10, 1961

This course is open to all grades of employed laboratory personnel who are approved by their State Health Officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, cytochemical techniques, and testing of drug susceptibility will be included. Demonstration and training in techniques for the isolation and identification of unclassified acid-fast bacilli will also be presented.

Laboratory directors and senior laboratory staff members may also make application for this course. No tuition or laboratory fees are charged. Reservations for this course should be made well in advance. Application forms may be obtained from the Laboratory Branch, Communicable Disease Center, Public Health Service, Atlanta, Georgia.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant—TERRI MANN

VOLUME 82 July 1960 ABST. NO. 1

The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 207 journals which represent 31 countries and which are published in 18 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Bronchial Diverticulum. Report of a Case and Review of the Japanese Literature (in Japanese). E. YOKOYAMA*, S. HATANO, and T. SHIRAISHI. *Clin Resp Organs*, November, 1959, 14: 901–906. (*Tokyo U. School of Med., Japan)

A case report is presented of a bronchial diverticulum seen in a 29-year-old female whose chief complaints were right-back pain and occasional bloody sputum. The diverticulum was located in the right main bronchus at the level of the orifice of the right upper lobe bronchus and opposite to it, and was confirmed by bronchoscopy and bronchogram. Its size was 8 mm. x 5 mm. The patient was conservatively treated and showed the same finding 2 years later.

Five other cases of bronchial diverticulum have been reported in Japan since 1951, 2 in males and 3 in females. Their ages were 31 to 58 years; 1 case was found at autopsy, another was complicated by pulmonary gangrene, and 3 cases were complicated by pulmonary tuberculosis. All of the diverticula were located medially in the right main bronchus 1.8 to 2.2 cm. from the carina. —I. TATENO

Cytologic Diagnosis of Bronchopulmonary Cancer (in Spanish). J. A. PILHEU* and O. CROXATTO. *Torax*, March, 1958, 7: 64–69. (*Buenos Aires, Argentina)

A series of 200 patients (196 males and 4 females) who had abnormal roentgenograms of the chest were studied to determine the presence or absence of bronchopulmonary cancer. The tumors were localized as follows: right, 112; left, 55; carina, 9; trachea, 9; undetermined, 15. The bronchoscopic findings were: endobronchial tumor, 128; typical funnel sign, 21; extrinsic pressure, 18; stenosis and parietal fixation, 8; mucosal infiltration, 6; atypical mucosal ulceration, 2; purulent secretions, 4; normal appearance, 18; mixed findings, 5. The results of 130 bronchoscopic biopsies were: epidermoid carcinoma, 75; undifferentiated carcinoma, 22; "oat cell" tumor, 16; adenocarcinoma, 3; unclassified, 3; negative, 11. The results of 93 selective bronchial washings performed at the time of bronchoscopy were: neoplastic cells, 47; very suspicious cells, 39; suspicious cells, 7.

In 71 cases in which bronchoscopy did not reveal a tumor, but showed other signs (compression, funnel sign, ulceration, et cetera), selective bronchial lavage was positive in 49, and in 11 the combination of biopsy and lavage was diagnostic, leaving only 11 cases undiagnosed. Biopsy alone yielded positive results in 60 per cent, and bronchial lavage with cytologic examination, in 43 per cent. The combination of these two techniques yielded a positive diagnosis of cancer in 89.5 per cent of the 200 cases studied. In the 21 patients who could not thus be diagnosed, biopsy of the cervical lymph nodes and thoracotomy were required.—F. PEREZ PINA

Correlation between Respiratory Diseases and Cor Pulmonale (in Spanish). V. BOZAL URZAY*. *Enferm Torax*, No. 29, 1959: 73–81. (*Dispensario de Palencia, Spain)

Cor pulmonale was diagnosed in 350 (5 per cent) of 7,000 patients with various pulmonary diseases. It was most frequently associated with the following respiratory processes: infectious diseases (pneumonia, bronchitis, lung abscess); diseases of long duration with parenchymal involvement (emphysema, bronchiectasis, broncholithiasis); the pneumonoconioses; tuberculosis; anatomic alterations of the chest (kyphoscoliosis); and tumors, in that order. It is noteworthy that 92 of the 350 patients had normal electrocardiograms. —Z. VIRÁGH

Hydatid Cyst of the Thymus—First Reported Case? (in Spanish). V. A. UGON*, S. PIOVANO, and D. TOMALINO. *Torax*, March, 1959, 8: 48–52. (*Saint Bois Colony, Argentina)

What is believed to be the first reported case of a primary hydatid cyst of the thymus is described. The patient was a 14-year-old Argentinian boy who, 5 years previously, had had a thoracotomy for excision of a hydatid cyst of the left lung. He was referred again because of the finding of a mass in his chest during a school roentgenographic survey. Physical examination showed the old thoracotomy scar, but was otherwise normal. Roentgenograms of the chest showed a left hilar mass and, superiorly, a smaller additional mass near the aortic knob. The heart and lungs were otherwise normal. On fluoroscopy, the masses did not pulsate. The electrocardiogram was normal, sputum was negative, and the hemogram was normal. The Casoni reaction was positive.

At operation, there were easily dissected adhesions of the left lung to the thoracic wall and to the mediastinum. The lung appeared healthy and showed a walnut-sized nodule superiorly, which was excised. It was a hydatid cyst containing small vesicles. Another tumor the size of a hen's egg was noted within a hypertrophied thymus. The thymus with the cyst was removed and the latter was found to contain watery fluid. Microscopic study of this tissue showed, in effect, that this was thymus and was unusually well pre-

served for the patient's age. The hydatid cyst was actually within the thymic tissue. The patient made an uneventful recovery.—F. Perez Pina

Mediastinal Echinococcosis (in Spanish). E. Trigo, D. L. Perazzo, and N. Carnovali. *Torax*, March, 1959, 8: 32–47.

A review of the literature of mediastinal echinococcal cysts and a case studied by the authors are presented. The patient discussed is a 20-year-old native Argentinian of rural origin. A pulmonary shadow was found on a survey chest roentgenogram. A brother had had a hydatid cyst of the scalp. The patient felt perfectly well and physical examination was negative. The chest roentgenogram showed a spherical tumor in the right hemithorax, appearing to bulge out of the mediastinum, contiguous to the right superior cardiac border, measuring in the lateral view about 8 x 5 cm., wider in its antero-posterior axis. Bronchograms showed poor filling of the bronchi corresponding to the anterior segment of the right upper lobe. The hemogram was normal, with 3 per cent eosinophils; the sedimentation rate was 2 mm./hr; the Casoni skin test, the Ghedini reaction, and the Kahn test were negative. The preoperative diagnosis was hydatid cyst of the mediastinopulmonary region. Thoracotomy was performed and the mediastinal tumor was completely excised. On gross section it was found to be a unilocular hydatid cyst, and laboratory studies revealed scolices in the fluid. The postoperative course was uneventful and the patient was discharged on the 15th postoperative day. A detailed review is made of 15 proved cases of mediastinal echinococcosis reported in Argentina.
—F. Perez Pina

Pulmonary Edema in Acute Heroin Poisoning. Report of Four Cases. R. Silber* and E. Clerkin. *Amer J Med*, July, 1959, 27: 187–192 (*N. Y. U. College of Med., N. Y., N. Y.)

Four cases are reported of pulmonary edema secondary to acute heroin poisoning. A period of coma ranging from "a few minutes" to "one hour in length" followed the self-injection of heroin. Cyanosis, dyspnea, orthopnea, and apprehension were apparent in all 4 patients when they regained consciousness. There was a cough productive of white mucoid- or blood-tinged sputum. Upon hospital examination, the respiratory rate was found to be rapid in all of the patients, although severe respiratory depression was described prior to admission. Although tachycardia was present in every case, the blood pressure remained within normal limits. A mild febrile response was noted in 2 patients. Distension of the neck veins with filling from below was apparent in 1 case, whereas hepatomegaly, gallop rhythm, and ankle edema were never observed. Auscultation of the lungs revealed the presence of widespread rales, variable in distribution. Glycosuria was present in 2 patients, a finding not unusual in morphine poisoning. The roentgenographic manifestations were compatible with the diagnosis of pulmonary edema and had resolved when a follow-up film was taken 16 to 80 hours later.

As can be seen from this description, the only distinguishing features of a patient with this form of pulmonary edema are a history of opium alkaloid administration or the finding of an obtunded sensorium, miotic pupils, and/or multiple venipuncture scars on physical examination. The pathogenesis of heroin-induced pulmonary edema is not clear. It is a manifestation of acute opium alkaloid toxicity.—T. H. Noehren

Results of Lactic Dehydrogenase Determinations in Benign and Malignant Effusions. M. V. de Torregrosa*. *Amer J Med Sci*, November, 1959, 238: 552–556. (*U. of Puerto Rico School of Med., San Juan)

Comparative studies of serum and effusion lactic dehydrogenase levels seem to be of great help in the differentiation of benign and malignant conditions. In the great majority of patients with untreated carcinomatosis, lactic dehydrogenase values in the effusions were higher than the serum values. In contrast, the pleural and ascitic fluids in patients with miscellaneous benign conditions (cirrhosis, cardiac failure, collagen diseases, and tuberculosis) had a lower content of lactic dehydrogenase than was found in the serum. Among 3 patients suffering from active pulmonary tuberculosis with pleural effusion, one had a higher value for lactic dehydrogenase in the effusion than in the serum.—W. J. Steininger

A Hospital Study of Congestive Heart Failure. Physicians of the Sheffield Hospital Region*. August 22, 1959, No. 5146: 201–208. (*Sheffield, England)

Among 487 cases of cardiac failure, 172 (35 per cent) were due to cor pulmonale, 167 (34 per cent) to degenerative heart disease, and 106 (22 per cent) to rheumatic fever. Among the patients with cor pulmonale, the ratio of males to females was 3.5 to 1. The over-all death rate of 35 per cent was similar in the various groups. Bronchitis and/or emphysema was the underlying chest disease in more than 96 per cent of the patients with cor pulmonale. Bronchiectasis was noted in 30 patients and kyphoscoliosis was present in 12. Pneumoconiosis, tuberculosis, pulmonary fibrosis, and

pulmonary embolism were rare causes. Recurrent attacks of bronchitis were more than 3 times as common in the cases of cor pulmonale than in either of the other groups. A history of anginal chest pain was obtained from 11 patients (6 per cent) with cor pulmonale. A history of mucopurulent sputum was more than 4 times as common in the cases of cor pulmonale than in the other groups. A high incidence of cyanosis of the lips (93 per cent) and of the tongue (83 per cent) was noted. A drowsy or mentally disoriented state was present in 40 per cent of the patients with cor pulmonale and was twice as common as in the other groups. Auricular fibrillation was present in 9 patients. A "P pulmonale" wave and right ventricular strain pattern were the chief electrocardiographic abnormalities. Physical signs and roentgenographic evidence of emphysema were present 4 times as frequently among the cases of cor pulmonale than among the cases of degenerative heart disease. Signs of bronchospasm predominated among this group.

The distribution of hematocrit values was remarkably similar in the degenerative and combined rheumatic and miscellaneous cases, about half of whom had values which lay between 40 and 50. The hematocrits of the cor pulmonale cases were often higher, and 57 per cent of results fell between 45 and 55 per cent. Twenty-seven per cent of the cor pulmonale cases had hematocrits in excess of 55 per cent, compared to only 9 per cent of the rheumatic and miscellaneous cases and 2 per cent of the degenerative cases. The blood urea nitrogen was elevated in 59 per cent of the cases. The blood alkali reserve was found in excess of 70 volumes per cent in more than half of the cases. A high number of patients with cor pulmonale lived in industrial areas and a high proportion of heavy smokers was noted among the cases of both cor pulmonale and degenerative heart disease.
—E. A. Riley

Spontaneous Idiopathic Hemopneumothorax (in Spanish). N. Gonzalez de Vega*, C. Gomez-Moreno, and M. Rodriguez Aguilar. *Enferm Torax*, No. 31, 1959: 223–234. (* Dispensario Antituberculoso Central, Granada, Spain)

The case history is presented of a 28-year-old female who had had pneumonia on the right side one year previously. By physical examination and fluoroscopy, a diagnosis of right spontaneous hemothorax was made and confirmed by thoracentesis. No pneumothorax was found. In about 2 months, 6 thoracenteses were done and the fluid obtained was grossly bloody; but, when streptokinase and streptodornase were instilled, the effusion gradually became serofibrinous. Six months from the onset, only a rather wide pleural thickening remained. No pulmonary disease was found that could have been suspected as an explanation for the hemothorax. It is noteworthy that the blood in the chest cavity did not show any clotting.

A complete review of the literature is presented: 219 cases are described, 15 of them female. This is the first such case reported from Spain.
—Z. Virágh

Idiopathic Pulmonary Hemorrhage in Pregnancy: Report of a Case Suggesting Early Pulmonary Hemosiderosis with Clinical Recovery after Steroid Therapy. V. Gurewich* and M. A. Thomas. *New Engl J Med*, December 3, 1959, 261: 1154–1159. (*MacDill Air Force Base, Tampa, Florida)

A case of idiopathic pulmonary hemorrhage (so-called idiopathic pulmonary hemosiderosis) is presented; this is the first case reported during pregnancy, and the seventh found in the literature in an adult more than 20 years of age. The patient was gravely ill with cough, breathlessness, cyanosis, and a hemoglobin of only 4.8 gm. per 100 ml. Roentgenographic study of the chest showed a massive, mottled infiltration of both lung fields, tending to spare the apexes and the costophrenic sulci. Because of the characteristic clinical picture and the absence of any other cause of blood loss, the diagnosis of idiopathic pulmonary hemorrhage was tentatively made, and appropriate treatment was instituted. Of particular interest is the fact that complete remission rapidly followed the use of steroids in this critically ill patient.

A biopsy of the lung was obtained on the tenth hospital day after 5 days of steroid therapy had been followed by striking clinical improvement and almost complete resolution of the infiltrations on roentgenogram. Microscopic examination of the biopsy showed blood within the alveoli, but not the marked hemosiderosis characteristic of idiopathic pulmonary hemosiderosis. Whether essential acute pulmonary hemorrhage and idiopathic pulmonary hemosiderosis are separate entities, or whether essential pulmonary hemorrhage may be the precursor of the other condition, are discussed. It is suggested that, in view of the possible life-saving role of steroids, the diagnosis of essential acute pulmonary hemorrhage or idiopathic pulmonary hemosiderosis be considered in every case of unexplained anemia associated with a diffuse pulmonary infiltration.—M. J. Small

Transposed Pulmonary Veins (in Hungarian). F. Horváth* and Gy. Balogh. *Tuberkulozis*, March, 1959, 3: 60–64. (* U. of Budapest, Hungary)

This disorder is a developmental anomaly. It might be a total transposition of all of the pulmonary veins (in which event life is not possible) or it may happen that only some of them open into

the right atrium, either immediately or by way of the superior or inferior vena cava. The condition may present symptoms such as dyspnea, a feeling of pressure around the heart, and easy fatigue. Three cases are reported in which the anomaly was recognized by conventional roentgenograms showing a ribbon-like vertical shadow in the middle lung field on the affected side. Angiograms done in one patient were not contributory.
—Z. VIRÁGH

Differential Diagnosis of Middle Lobe Syndrome (in German). W. SIEGENTHALER* and P. KAEGI. *Schweiz Med Wschr*, August 29, 1959, 89: 915–920. (*Universitatspoliklinik, Zurich, Switzerland)

Nine cases of middle lobe syndrome and 1 case of lingula syndrome were diagnosed among 13,640 patients in an outpatient clinic. The ages of these patients (6 male and 4 female) varied between 40 and 78 years. They were sent to the clinic because of febrile bronchopulmonary infections or incidental finding of a pulmonary infiltration. In no instance was the syndrome diagnosed before the examination in the clinic.

Of 4 cases of acute middle lobe syndrome, there were 3 with primary bacterial pneumonia and 1 with ornithosis pneumonia. The picture is that of an acute infectious disease with complete restitution. The middle lobe does not shrink. During the infectious phase there is bronchoscopically only inflammatory swelling (sometimes with a layer of pus in it) in the mucous membrane of the middle lobe. The case of lingula syndrome belongs also to the group of acute infections and, specifically, ornithosis infection. In contrast to the acute form, which does not show any special characteristics, the chronic form of the middle lobe syndrome has characteristics which depend on the special features of the middle lobe bronchus.

The etiology of the 5 chronic cases was tuberculosis in 2 instances, benign lymphogranulomatosis in the hilar lymph nodes in 1, anthracosis of the lymph nodes in 1, and bronchitis deformans in 1. In 3 cases there were symptoms of chronic bronchitis or recurrent pneumonic attacks. In 2 cases the findings were negligible. The differential diagnosis can be made only on the basis of clinical symptoms, bronchoscopy with biopsy, bacteriologic examination of bronchial secretion, and roentgenography. In all chronic cases, bronchoscopy revealed a stenosis or occlusion of the middle lobe bronchus or one of its ramifications. In 2 cases a perforation was visible. Because the chronic form extends over months or years, the irreversible atelectasis is followed by a shrunken middle lobe or a destroyed middle lobe if the parenchyma practically doesn't exist and only the ectatic bronchi are left.—J. HAAPANEN

The Systemic Mycoses. M. LITTMAN*. *Amer J Med*, July, 1959, 27: 1–3. (*Mount Sinai Hosp., N. Y., N. Y.)

Improvements in laboratory diagnostic methods have progressed to the point where the majority of pathogenic fungi can be isolated directly from a patient's exudates within 3 to 6 days. Culture of the gastric aspirates of patients with pulmonary mycotic infections is especially valuable for recovering the etiologic agent. Instead of using the slow animal technique of inoculation of white Swiss mice with exudates, followed by cultivation of the animal's liver and spleen on peptone agars—a process that may take from 3 to 6 weeks—liver-spleen-glucose-blood agar has been successfully employed for the rapid direct isolation of pathogenic fungi at 37°C.

With the discovery of amphotericin B (Fungizone®) and its use as an intravenous medication, treatment of the systemic mycoses has been greatly improved. The consensus of investigators is that amphotericin B is highly effective for disseminated mycoses such as North and South American blastomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis, and moniliasis. The clinical effectiveness of amphotericin B for the systemic mycoses is limited by the rapidity with which susceptible pathogenic fungi become resistant to the antimicrobial. The current wide use of nystatin together with broad-spectrum antimicrobials as a prophylactic to prevent monilial overgrowth in the gastrointestinal tract during antimicrobial therapy may very well lead to the increased resistance of *C. albicans* and other pathogenic fungi to both nystatin and amphotericin B. This could be disastrous for patients with disseminated mycoses, since amphotericin B is the only really effective medication available at the present time.—T. H. NOEHREN

Bronchial Aspergillosis. A Clinical Study with Anatomic and Histologic Findings (in Italian). U. AGOSTNI*. *G Ital Tuberc*, July–August, 1959, 13: 229–236. (*Osp. S. Giovanni, Venice, Italy)

Only about 10 cases of bronchial aspergillosis have been definitely diagnosed in Italy. One case in an 18-year-old girl is briefly described both clinically and anatomically after lobectomy was performed. No fungus was isolated, but the anatomic and histologic lesions substantiated the clinical diagnosis.—I. ARCHETTI

Late Progression of Pulmonary Coccidioidomycosis. M. J. SMALL*. *AMA Arch Intern Med*, November, 1959, 104: 692–740. (*V. A. Hosp., East Orange, New Jersey)

Four cases have been observed within the last 2 years which strongly tend to indicate that

pulmonary coccidioidomycosis, contrary to the commonly held current concepts: (*1*) may be a disease which does not manifest itself until a prolonged period after exposure in the endemic area; (*2*) may undergo late, continued, slow progression over a period of years; and (*3*) may spread by the bronchial route from cavities previously considered benign to produce infiltrations years later in the lung, indistinguishable by roentgenogram from those of exudative tuberculosis. All of these cases were observed in persons who resided in the eastern part of the country and whose roentgenographic manifestation of disease did not become manifest until 5 months to many years after their documented residence in a known epidemic area for coccidioidomycosis.

Thus, it would appear that late pulmonary manifestations of coccidioidomycosis must be added to the list of differential diagnoses when considering stationary or progressive coin lesions of the lung, cavitary lesions without positive bacteriology for tuberculosis, and parenchymal infiltrations resembling tuberculosis, even years after exposure in an endemic area. A carefully taken history eliciting residence in the endemic area, as well as use of the coccidioidin and tuberculin skin tests, have been the most helpful factors in leading to a positive diagnosis of late, active, pulmonary coccidioidomycosis. With the increasing tendency toward vacations by flight to the southwestern part of the country, it is believed that physicians in all parts of the country must be alert to this possibility.
—W. J. STEININGER

Disseminated Cryptococcosis Producing a Sarcoid Type Reaction. L. H. SHIELDS*. *AMA Arch Intern Med*, November, 1959, 104: 763–770. (*2008(B) N. John Russell Circle, Elkins Park, Penn.)

An unusual case of disseminated cryptococcosis presented with symptoms of acute polyarthritis. Although the diagnosis of cryptococcosis was established, many of the aspects of the disease pattern suggested sarcoidosis. Clinical remission was induced by the use of intravenous amphotericin B. Laparotomy 13 months after initiation of therapy showed involvement of the liver, spleen, and regional lymph nodes by a typical sarcoid-like reaction. Cryptococcal organisms were found in some of the granulomas. The clinical status of the patient in remission is indistinguishable from that seen in typical Boeck's sarcoidosis. It is believed that the widespread sarcoid-type reaction observed represents an immune response to primary cryptococcal invasion. The case is unusual in that the infective cryptococci are not only associated with sarcoidosis, but are actually demonstrated in the area of sarcoid-type response, supporting the concept that the sarcoid reaction may be caused by various etiologic agents.
—W. J. STEININGER

The Course and Prognosis of Histoplasmosis. H. RUBIN*, M. RUBIN, M. FURCOLOW, J. YATES, and C. BRASHER. *Amer J Med*, August, 1959, 27: 278–288. (*U. of Kansas Med. School, Kansas City, Kansas)

An analysis is presented of 123 culturally proved cases of histoplasmosis. The cases have been divided by clinical types and analyzed separately. Several pertinent observations concerning the prognosis of this infection emerge from the data: Acute pulmonary histoplasmosis is a benign infection in most instances. Although the number of such cases included in this compilation are small, they were all clinically severe, and all of the patients are now in good health. Acute progressive histoplasmosis, often referred to as disseminated histoplasmosis, has a poor prognosis. There is clinical evidence of dissemination beyond the lungs to other organs. Included in this group are patients with seemingly isolated mucous membrane lesions. Such cases are usually fatal within one year, although an occasional patient does survive. Chronic pulmonary histoplasmosis is characterized by cavities in the upper lobes. This form of the disease characteristically progresses gradually over several years, although remissions and exacerbations may occur. The prognosis is poor if the patients are not treated (Authors' summary).—T. H. NOEHREN

The Protean Manifestations of Histoplasmosis as Illustrated in Twenty-Three Cases. F. G. CONRAD*, S. SASLAW, and R. J. ATWELL. *AMA Arch Intern Med*, November, 1959, 104: 692–709. (*Ohio State U. College of Med., Columbus)

Twenty-three cases of histoplasmosis presented diagnostic problems with a variety of manifestations: 7 had acute disease resembling common respiratory infections; 8 had chronic debilitating pulmonary disease resembling tuberculosis (5 of these were treated for tuberculosis and 3 actually had concurrent or past cultures of *M. tuberculosis*); 1 patient presented with multiple arthralgias and weakness, was thought to have sarcoidosis, but was shown to have disseminated histoplasmosis; 1 presented as a classical hypersplenism and died with cerebral vascular hemorrhage secondary to thrombocytopenia. Treatment is mainly supportive. Surgery may be indicated for single localized lesions. Amphotericin B

systemically shows promise but, because of its side reactions, should be reserved for selected cases.—W. J. STEININGER

Evaluation of Lipoid Pneumonia. R. H. SUNDBERG*, K. E. KIRSCHNER, and M. J. BROWN. *Dis Chest*, December, 1959, 36: 594–601. (*Mercy Hosp., San Diego, Calif.)

Granulomas of the lung due to lipoid substances have been recognized for many years. They are believed to fall into two types: (*1*) exogenous, manifested by paraffinomas or oil granulomas due to aspiration of mineral, vegetable, or animal oil; and (*2*) endogenous (cholesterol pneumonitis, Wegener's granulomas, et cetera), resulting from metabolic, allergic, neoplastic, inflammatory, embolic, or toxic reactions within the lung tissue. Those lesions of exogenous nature may be diagnosed by a careful history (coughing and wheezing, usually nocturnal, are the prominent symptoms, along with repeated colds which do not respond to the usual therapy), and repeated sputum analyses and special stains. Removal of the offending oil almost always is sufficient for complete relief.—E. A. ROUFF

Lobar Pneumonia with Bacteremia Caused by *Bacterium Anitratum*. L. GLICK*, G. MORAN, J. COLEMAN, and G. O'BRIEN. *Amer J Med*, July, 1959, 27: 183–186. (*Loyola U., Chicago, Illinois)

A severe case of lobar pneumonia and bacteremia caused by *B. anitratum* is presented together with a review of the literature on this organism and its bacteriologic characteristics. *Bacterium anitratum* is a large (0.8 μ) gram-negative Diplococcus which is encapsulated and has a pleomorphic nature. Specific strains may be either motile or nonmotile. On agar it is characterized by large white or buff mucoid colonies. Biochemically it is capable of reducing only the simpler carbohydrates of 5 carbons or less. Because of its inability to reduce nitrates, it was christened anitratum. This organism, as well as all members of the Mimeae tribe, mimic *Neisseria gonorrhoeae* to such a degree that differentiation on smears is impossible. The consequent hazard of diagnosis by smear in genitourinary tract disease has been pointed out by Ewing and DeBord.

The fact that *B. anitratum* has been infrequently reported in the past bespeaks its low pathogenicity. The majority of workers consider it to be an unusual saprophyte. Therapeutic experiences in general have shown the tetracyclines to be the most efficacious in treating infections caused by *B. anitratum*. The present case calls attention to the fact that unknown organisms may be encountered with increasing frequency as virulent pathogens. This is due in part to the widespread use of antimicrobials which have tended to change the previous pathogenic flora.—T. H. NOEHREN

Pneumocystis Carinii Pneumonia: Report of a Case Diagnosed during Life. J. HENDRY* and R. F. MYERS. *Canad Med Ass J*, November 15, 1959, 81: 831–835. (*Brandon General Hosp., Manitoba, Canada)

A further case is reported of *Pneumocystis carinii* pneumonia diagnosed during life, which would appear to be the 26th reported in Canada. Attention is drawn to the paucity of organisms seen and the scanty alveolar "honeycombing," even though the other microscopic features and gross clinical findings were typical of the condition. The diagnosis was considered clinically because of the roentgenographic appearance and the marked cyanosis and air hunger associated with minimal, if any, local signs of pulmonary involvement.—E. A. RILEY

Primary Chickenpox (Varicella) Pneumonia: Report of Two Cases Seen in the Private Practice of Internal Medicine. J. GABLE, JR.* *Ann Intern Med*, September, 1959, 51: 583–590. (*Oklahoma City Clinic, Okla.)

Primary chickenpox pneumonia is usually a disease of adults. It has been reported with increasing frequency in the last few years. Two additional cases encountered in the private practice of internal medicine are reported.—T. H. NOEHREN

Primary Varicella Pneumonia. O. MCCARTHY*. *Brit Med J*, November 28, 1959, No. 5160: 1155–1156. (*Royal Naval Hosp., Chatham, England)

A case of varicella pneumonia occurring in a 26-year-old male is reported. This is the first instance of this condition reported in England.—E. A. RILEY

"Allergic Pneumonitis." R. P. MCCOMBS*. *Canad Med Ass J*, November 1, 1959, 81: 703–706. (*Pratt Diagnostic Clinic, Boston, Mass.)

Through a study of 11 patients in whom clinical, physiologic, and pathologic data were correlated, it became apparent that pneumonitis, probably due to an allergic mechanism, occurs fairly commonly in the course of several systemic diseases or as a direct allergic reaction in the lung following parenteral administration of a drug or antimicrobial. The course is subacute or chronic, and in some cases may eventually result in pulmonary fibrosis, chronic respiratory failure, and cor

pulmonale. Since there may be other systemic manifestations, prognosis is often determined by these, especially if there is renal involvement. The primary pathologic lesion in the acute stage is inflammatory and localized in and about small blood vessels. In the chronic stage the lesion is fibrotic. Recognition of this disorder is important because therapy with corticosteroid drugs alleviates many of the respiratory symptoms and may prevent advanced pulmonary fibrosis. It is important to rule out bacterial infection (Author's summary).—E. A. RILEY

Asian Influenzal Pneumonitis. P. SOTO* and J. WYATT. *Amer J Med*, July, 1959, 27: 18–25. (*St. Louis U. School of Med., Missouri)

Nine patients with fulminant cases of Asian influenzal pneumonia were examined by pathologic and virologic studies. The diagnosis in 6 of the 9 cases was proved by isolation of the Asian strain of the influenza virus from the lungs, the remainder by the distinctive histologic findings.

The establishment of a correct diagnosis in an epidemic is of the greatest importance, and numerous means are available to accomplish this. Included are serologic, virologic, and bacteriologic studies, both pre- and post mortem, as well as necropsy examination. If a necropsy cannot be performed, one technique of interest, easily performed, is to obtain a needle specimen of the lungs immediately after death to obtain tissue for cultures and microscopic study. Such a technique should be more widely used in establishing a diagnosis in any further respiratory disease epidemics such as the recent one of Asian influenza. The material garnered contributes variable information on the specificity of lesions, the extent of the disease in the general population and, possibly, the existence of the virus in the lungs without the existence of histopathologic alterations in individuals dying from some other cause.

Anoxemia was thought to be the essential cause of death. The anoxemia was due to a mechanical alveolar-capillary block. Biophysical analysis by ultra-soft X-rays indicates the density of the hyaline membrane, offering a mechanical block to gaseous exchange. Contributory causes were bacterial superinfection and the generalized toxemia.—T. H. NOEHREN

Two Cases of Nonspecific Chronic Pneumonitis Simulating Lung Cancer (in Japanese). M. KUSAGAWA*, C. OKUMOTO, M. YAMADA, I. HATTORI, and T. NISHIKAWA. *Jap J Thoracic Surg*, November, 1959, 12: 1039–1043. (*Mie Prefectural U. School of Med., Japan)

Two cases are reported of nonspecific chronic pneumonitis simulating lung cancer. The differential diagnosis between the two diseases was very difficult, and the diagnosis was actually made postoperatively by histology. Early thoracotomy is recommended in suspected cases. Three features are pointed out as rather characteristic of this disease: comparatively good physical condition (although 1 patient, a 67-year-old male, was anemic, with 65 per cent hemoglobin and a 2.37 million per cu. mm. erythrocyte count); well-defined and localized shadow in a lobe or in any segment of the lung; and the histologic feature of marked fibrosis with extensive infiltrations of lymphocytes and plasma cells. One patient, aged 67, had a history of about one month's cough, sputum (occasionally bloody), and right-chest pain. Another patient, a 33-year-old female, had been treated for pulmonary tuberculosis.
—I. TATENO

Some Observations on the Clinical Picture and Therapy of Putrid Pulmonary Processes, Based on About 200 Cases Observed and Treated. J. SWIERENGA*. *Dis Chest*, December, 1959, 36: 579–590. (*St. Anthony Hosp., Utrecht, Holland)

A "putrid pulmonary process" means an inflammatory process of the lung in which one or more abscess cavities have developed; consequently, it is not an inflammation in a preformed cavity. The gross etiologic classification defines: (*1*) putrid bronchogenic process, (*2*) metastatic pulmonary abscesses, (*3*) putrid pulmonary processes produced by spread from an adjacent organ and, (*4*) putrid pulmonary processes in conjunction with a pulmonary infarct. The causative organism may be practically any aerobic or anaerobic bacteria. Treatment through the years has included systemic chemo- and antimicrobial therapy, aerosol medication, bronchoscopic instillation of drugs, external drainage, and surgical resection. Excellent results were obtained in the present series after the introduction of combined general and local antimicrobial therapy in conjunction with the Metras catheter (direct introduction of this special catheter under fluoroscopic control into the bronchial segment after pharyngeal anesthetization, and instillation of penicillin and streptomycin; this is usually done daily). With this technique, 67 of 119 patients were cured; the remaining 52 patients required excision of the residual lesion.—E. A. ROUFF

Rheumatoid Pulmonary Disease: Report of a Case. F. J. FLATLEY*. *New Engl J Med*, November 26, 1959, 261: 1105–1108. (*Strong Memorial Hosp., Rochester, N. Y.)

This report concerns an investigation of a patient with rheumatoid arthritis, pneumonitis,

and recurrent pleural effusions. Microscopic examination of the surgically removed lung revealed granulomatous lesions indistinguishable from the typical rheumatoid nodules. It is believed that this represents a rare case of rheumatoid pulmonary disease. The patient was a 62-year-old, nonpneumoconiotic man. The clinical picture in the cases previously reported is widely variable. As in this case, pulmonary manifestations usually occur some time after the appearance of, or with recrudescence of, joint symptoms. Pulmonary changes before the onset of joint manifestations have not been reported in nonpneumoconiotic patients. Pleuritis is a constant finding. This may be mild and transient, recurrent or persistent, and manifested by pleurisy or effusion. More commonly, pulmonary symptoms accompany the pleural changes. The pulmonary symptoms may also be mild and transient or, more frequently, progressive with increasing dyspnea and incapacitation. Repeated exacerbations of pleuritis, dry cough, and fever unresponsive to antimicrobial therapy are common. Pulmonary hypertension may result, with the subsequent development of cor pulmonale.

The pathologic findings of pachypleuritis, interstitial and fibrinous pneumonitis, pulmonary fibrosis, and rheumatoid nodules in the lung parenchyma support the diagnosis. The distinctive pathologic finding is the rheumatoid nodule or granuloma. This consists of an inner zone containing either fibrinoid changes or necrosis and degeneration, an intermediate zone with proliferating cells often in regular, palisade arrangement, and a peripheral zone of inflamed tissues in which the granuloma appears.

In the case presented, rheumatoid pulmonary activity is believed to have extended, with remissions and exacerbations, over a 10-year span. At the present time, the patient has no pulmonary symptoms, and arthritic symptoms are controlled by minimal doses of corticosteroids. Early management of superimposed infections with appropriate antimicrobials and induced hyperadrenalism at the time of exacerbations appears now to be the treatment of choice of rheumatoid pulmonary disease; usually, there is no alteration in the roentgenographic appearance of the chest. —M. J. Small

Hypopituitarism Due to Hypothalamic Sarcoidosis. H. A. Selenkow*, H. R. Tyler, D. D. Matson, and D. H. Nelson. *Amer J Med Sci*, October, 1959, 238: 456–463. (*Harvard Med. School, Cambridge, Mass.)

Hormonal insufficiency of the anterior and posterior portions of the pituitary gland occurs rarely as a complication of sarcoidosis. This insufficiency is due almost always to granulomatous involvement of the pituitary gland itself. The patient described in this report exhibited the unique occurrence of panhypopituitarism due to extensive hypothalamic involvement with sarcoidosis with a histologically intact pituitary gland. This suggests that interruption of the neural pathways between the hypothalamus and the pituitary gland is capable of producing the syndrome of both anterior and posterior pituitary failure in man. Therapeutic administration of prednisolone failed to alter the clinical course of this patient's sarcoidosis.—W. J. Steininger

Sudden Death Due to Myocardial Sarcoidosis, with a Comment on the Etiology of Sarcoid. T. P. Poon* and W. D. Forbus. *AMA Arch Intern Med*, November, 1959, 104: 771–778. (*Duke U. Med. Center, Durham, N. C.)

Two cases are described of sudden death due to widespread myocardial sarcoid granuloma, bringing the total on record to 17. With full appreciation of the well-known reaction potential of the cells of the reticuloendothelial system and the complexity of the granulomatous inflammatory reaction which is the product of reacting reticuloendothelial cells, it is suggested that sarcoid granuloma may well be attributed to provocation of the reticuloendothelial system by some chemical substance or substances which are widely disseminated and in no sense peculiar to any one animate or inanimate agent.—W. J. Steininger

Immunoconglutinins in the Serum of Silicotic Patients (in Italian). B. Pernis*, G. Gambini, and M. Finulli. *Med Lavoro*, April, 1959, 50: 250–256. (*Clinica Lavoro, U. of Milano, Italy)

The immunoconglutinin titer in the serum of 66 silicotic patients without associated infections was detected by a slight modification of the technique used by Marks and Coombs. It was found that 88 per cent of the cases had a titer greater than the normal maximal value of 1:4, and that almost half of them showed a titer between 1:32 and 1:128. The results obtained in this study correspond to those found by Marks and Coombs in patients with evolutive rheumatic arthritis, and are considered a new proof of the hypothesis that immunologic processes have an important part in the pathogenesis of silicosis. —I. Archetti

Serum Proteins and Glycoproteins in Pulmonary Silicosis (in Italian). L. Buscarini* and F. Nicrosini. *Med Lavoro*, May, 1959, 50: 368–378. (*U. of Pavia, Italy)

The serum protein and glycoprotein patterns were studied in 29 patients with pulmonary silicosis in three different stages of development

in order to clarify the knowledge of the alterations and their possible relationship to the disease. It was found that hyperproteinemia and hyper-alpha-2-globulinemia were slightly more evident in patients in the third stage; hypergamma-globulinemia was present in all three groups. In the third stage of the disease, the serum glycoprotein pattern showed a progressive increase of carbohydrates bound with the total proteins and the alpha-2-globulins, and an increase in value of the ratio between the alpha-2-globulin glucides and the alpha-2-globulin. The value of the serum glycoprotein pattern is particularly emphasized as a mirror of the degree of evolution of the disease.—I. ARCHETTI

Clinical and Roentgenographic Observations on the Association of Silicosis with Scleroderma (in Italian). A. FRANCIA*, G. MONARCA, and A. CAVALLOT. *Med Lavoro*, August–September, 1959, 50: 523–540. (*U. of Turin, Italy)

Six cases of silicosis associated with scleroderma, all definitely proved both clinically and roentgenographically, were observed among about 1,000 patients. The clinical histories and the roentgenographic findings are given in 4 of the cases, as well as the gross anatomic results in another. The roentgenographic diagnosis of the disease and the mutual interrelationship between silicosis and scleroderma are discussed. The influence of the sclerodermic process in worsening the course of silicosis and favoring the development of tuberculous complications is particularly emphasized.—I. ARCHETTI

Chronic Pulmonary Suppuration. N. H. DEJANNEY* and O. BIGMAN. *Dis Chest*, December, 1959, 36: 602–612. (*Herman Kiefer Hosp., Detroit, Mich.)

The prolonged antimicrobial treatment of pulmonary suppurative disease must be stressed even though the impression is gained from the medical literature that surgical treatment is preferred and should be instituted early. It is, actually, not solely a medical or surgical condition. It should always be treated medically initially, and surgery considered secondarily after proper medical treatment has been used and fallen short of cure. Bronchoscopy is indicated in almost every case, and may be used both diagnostically and therapeutically. Antimicrobials should be chosen on the basis of sensitivity studies, preferably from the bronchial secretions. Bronchographic study should be made in every case; this is especially important as a reference point if there is a recurrence.—E. A. ROUFF

Acute Atrophy of the Thymus Induced by Adrenocorticosteroids: Observed Roentgenographically in Living Human Infants. A Preliminary Report. J. CAFFEY* and C. DI LIBERTI. *Amer J Roentgenol*, September, 1959, 82: 530–540. (*College of Physicians and Surgeons, and the Babies Hosp., N. Y., N. Y.)

Oral administration of adrenocorticosteroids to infants with enlarged thymuses caused rapid thymic atrophy. Rapid thymic regrowth and overgrowth occurred following stoppage of the steroid in 3 cases in which adequate follow-up observations were made. In 1 case of natural-stress thymic atrophy, regrowth and overgrowth occurred in the same fashion as in artificially induced thymic atrophy.

Thymic shrinkage by steroids enables one to visualize the real cardiac size contours and vascular images above the heart. Corticosteroid shrinkage of triangular widening of the mediastinum makes possible the differentiation of a conspicuous triangular right lobe of the thymus from atelectasis of the right upper lobe of the lung, segmental pneumonic consolidation of the right upper lobe, and loculated mediastinal pleurisy. Fluctuations in the size of the mediastinum observed during and after the oral administration of corticosteroids explain, at least in part, the causal mechanism for the great variations in the size of the mediastinum found commonly in roentgenograms of healthy and sick infants. It seems likely that natural-stress atrophy stimulates the pituitary–adrenal axis to the excess secretion of steroids, which produces acute thymic atrophy at first, followed by regrowth and overgrowth.—T. H. NOEHREN

Pulmonary Tuberculosis, Carcinomatosis, and Bacterial and Aspergillus Infections in One Patient. A Histopathologic Study (in Japanese). Y. TAJIMA*, I. YO, Y. INOUE, and C. BABA. *Clin Resp Organs*, December, 1959, 14: 957–962. (*National Nakano Sanatorium, Tokyo, Japan)

The writers present the case of 68-year-old man who died, after 7 months of illness, of pulmonary tuberculosis complicated by carcinomatosis metastasizing from a duodenal site, and bacterial and Aspergillus infections of the lung. Whether the carcinomatous and tuberculous lesions interfered with each other's growth was the point of particular interest in presenting this case. Areas were seen of atrophic cancerous cells or tissue dispersed in tuberculous necrotic tissue, atrophic cancerous tissue with destructed adenomatous structure adjacent to tuberculous lesions, or areas of well-developed cancerous tissue encroaching on the tuberculous granuloma. These

histologic changes were dependent upon whether the blood supply to those areas was sufficient. It was poor in the first two and sufficient in the third type of lesions. The preponderance of one type of lesion over another was, therefore, not due to the basic character of each type of lesion. The growth of *Aspergillus fumigatus* was poor where tubercle bacilli were abundant, but the fungi grew well in the areas in which gram-positive bacilli were also abundant. It was rich both in the necrotic tissues or in the areas to which blood supply was poor.—I. TATENO

Eosinophilic Granuloma Confined to the Lung. Report of a Case. C. L. MENGIS*. *AMA Arch Intern Med*, October, 1959, 104: 580–584. (*San Juan Clinic, Farmington, New Mexico)

Because symptoms are minimal or absent, diagnosis of pulmonary histiocytosis (eosinophilic granuloma) in the general population is not likely unless routine survey films of adults are taken. The true incidence, as mirrored by the growing number of microscopically substantiated case reports, is probably larger than previously realized. An additional case, confined to the lung and occurring in a 28-year-old male, is reported. Prednisolone therapy resulted in little or no roentgenographic change.—W. J. STEININGER

Primary Lymphosarcoma of the Lung. E. R. HALL, JR.*, and B. BLADES. *Dis Chest*, December, 1959, 36: 571–578. (*George Washington School of Med., Washington, D. C.)

Primary lymphosarcoma of the lung is rare, the 3 cases here reported bringing up to 30 those noted in the literature. As with gastric lymphosarcoma, it may remain confined to the lung for a relatively long period, enlarging by direct extension. Vascular invasion is infrequent; however, distant metastases presumably occur by this route. The prognosis in resectable lesions is more promising than in bronchogenic carcinoma. The use of chemotherapeutic agents and/or roentgen therapy cannot as yet be properly evaluated.
—E. A. ROUFF

Tracheobronchial Papillomatosis Treated by Roentgen Irradiation. Report of Two Cases. J. BLACKMAN*, S. T. CANTRIL, P. K. LUND, and D. SPARKMAN. *Radiology*, October, 1959, 73: 598–606. (*Swedish Hosp., Seattle, Wash.)

Two cases of tracheobronchial papillomatosis are presented in which papillomatous masses obstructing the air passages responded favorably to 2,000-kv roentgen therapy. Such therapy is not applicable to the treatment of papillomas in the smaller bronchi and infiltrating the parenchyma of the lung, but is limited rather to the larynx, trachea, and bronchi within the mediastinum. From the standpoint of radiation therapy, the dose delivered to the trachea and main stem bronchi borders on that utilized in bronchogenic cancer. It is believed that a lesser dose would be ineffective. Although tracheobronchial papillomatosis is pathologically benign, the ultimate outcome is precarious in view of the chronicity of obstruction and attendant complications.
—W. J. STEININGER

TUBERCULOSIS, PULMONARY

Report on the Care and Treatment of the Tuberculous Alcoholic (in German). K. L. MERKEL*. *Wien Med Wschr*, April 25, 1959, 17: 359–364. (*Laab im Walde, Austria)

A questionnaire sent to 117 Austrian tuberculosis institutions—sanatoriums, tuberculosis wards in general hospitals, and tuberculosis dispensaries—with questions referring to tuberculous alcoholics resulted in responses from all but 7. Ninety per cent of the answers contained the opinion that the results of treatment are worse in alcoholic than in nonalcoholic patients, the causes being: short-term treatment, continued alcohol consumption, generally worsened condition, and additional organic damages. According to 1 investigation, ⅔ of 300 alcoholics terminated treatment against medical advice; 66 per cent of them left the hospital with positive bacillary finding. Another institution (the Laab im Walde) reported that almost 30 per cent of the alcoholic patients had gastric resection. In the same hospital, a relapse rate of 58 per cent was reported in the alcoholics as compared to 6 per cent in other patients during a period of 2 years.

Eighty-four per cent of the doctors reported that the atmosphere of the tuberculosis institution is unfavorably affected by the presence of the alcoholic. An emphatic "yes" (96 per cent) was the answer to the question whether the tuberculous alcoholic endangers his environment, especially the family group; consequently, 85 per cent of the doctors think that the tuberculous alcoholic should be treated in a special institution. However, the present laws of Austria enforcing the isolation of tuberculous alcoholics are not sufficient.—Z. VIRÁGH

Tuberculous Bronchocele (in French). P. GALY*. *Schweiz Z Tuberk*, No. 1, 1959, 16: 16–22. (*Hôpital Edouard-Herriot, Lyon, France)

This disorder is a sacculated bronchiectasis of a distal bronchus filled with caseous material. Tomographic and gross anatomic pictures are presented in illustration.—Z. VIRÁGH

Contribution to the Differential Diagnosis of Carcinoma and Pulmonary Tuberculosis (in French). M. TADDEI*. *Schweiz Z Tuberk*, No. 1, 1959, 16: 33–38. (*Sanatorium d'Humilimont, Marsens/Fribourg, Switzerland)

A 43-year-old male patient was treated with streptomycin, isoniazid, and PAS for an opacity in the right upper lobe. The right hilum was also enlarged. Tubercle bacilli were not demonstrated by routine sputum examinations that included gastric washing. A secretion removed by bronchoscopic aspiration was negative for acid-fast bacilli in guinea pigs. Bronchoscopy did not substantiate a previous diagnosis of neoplasm. The Mantoux test with 0.1 mg. of PPD was strongly positive. Scalene node biopsy (Daniel's method) rendered a diagnosis of tuberculosis with anthracosis. The lymph nodes removed by this biopsy were inoculated into guinea pigs and gave a negative reaction. The patient's clinical condition improved on chemotherapy, but the roentgenographic picture had not changed; therefore, thoracotomy was performed and, since the extemporaneous biopsy revealed adenocarcinoma, pneumonectomy was done. Some 6 months later the patient died of ascendant paralysis due to carcinoma.—Z. VIRÁGH

Corticotropin (ACTH) in the Treatment of Acute Tuberculous Pleural Effusion (in Russian). I. T. MALTSEV*. *Probl Tuberk*, No. 7, 1959, 47–50. (*Omsk Medical Institute, U.S.S.R.)

Twenty patients with acute tuberculous pleural effusions were treated with an average dose of 440 units of corticotropin intramuscularly in addition to specific chemotherapy. Marked improvement was noted on the second or third day after therapy was started. Temperature became normal, appetite returned, and tachycardia disappeared. The effusion resorbed within 7 days. Follow-up study from a few months to 4 years showed no reactivations. The effusion failed to clear in 7 of these patients while they were on chemotherapy alone, but disappeared after the hormone was added to the regimen. One patient with a hemorrhagic tuberculous effusion failed to respond to the treatment. The hospital stay of the patients was considerably shortened, and no complications were observed.—W. A. ZAVOD

Critical Study on Fistulization of Tuberculous Tracheobronchial Lymph Nodes (in French). J. M. LEMOINE*. *Schweiz Z Tuberk*, No. 1, 1959, 16: 23–32. (*189 Blvd. Saint-Germain, Paris, France)

Of 688 patients, mostly children with primary tuberculosis, 188 (38.5 per cent) had fistulas demonstrated by bronchoscopy. Among 2,051 tuberculous patients with chronic disease, there were 464 ulcerations or stenoses (22.6 per cent), but only 3 patients had fistulas. It is suggested that the depressions in the trachea and main bronchi seen endoscopically were not scars of a previous fistula but, rather, partial dystrophies of elastic and muscular elements of the mucosa. —Z. VIRÁGH

Associated Silicosis, Tuberculosis, and Bronchogenic Carcinoma (in French). P. GABUS*. *Schweiz Z Tuberk*, No. 1, 1959, 16: 10–15. (*Sanatorium "Beau Site," Leysin, Vand, Switzerland)

This case is believed to be the first reported in the literature on the coexistence of these three grave disorders; their presence was diagnosed during life and confirmed at autopsy. The patient died of tuberculosis.—Z. VIRÁGH

Pregnancy and Tuberculosis. G. W. THOMAS*. *Canad Med Ass J*, November 1, 1959, 81: 710–714. (*St. Anthony, Newfoundland, Canada)

Sixty-three tuberculous mothers with 228 full-term pregnancies and 16 miscarriages were studied. Nineteen of these women delivered while undergoing treatment for active tuberculosis, 35 had arrested disease, and 9 had tuberculosis untreated or inadequately treated during or after pregnancy. There were 3 deaths, an over-all mortality of 4.7 per cent, all occurring in the third group. The majority of patients were treated with a combination of surgery and chemotherapy. Four patients had relapses during pregnancy, a reactivation rate of 7 per cent among the adequately treated patients. It is believed that pregnancy has no influence on the course of tuberculosis in minimal and moderately advanced cases, and that therapeutic abortion has no place in the treatment of tuberculosis.—E. A. RILEY

Bronchial Involvement in Primary Tuberculosis (in Spanish). J. MÁRQUEZ-BLASCO*. *Enferm Torax*, No. 29, 1959: 25–57. (*Sanatorio Infantil de San Rafael, Segovia, Spain)

A long and detailed presentation.—Z. VIRÁGH

TUBERCULOSIS, NONPULMONARY

Recent Aspects of Tuberculous Meningitis: Report of Four Cases with Psychotic Disorders (in Japanese). N. OKUMURA*, H. KAMINAGA, T. AOYAMA, T. ICHIYAMA, and I. DEMIYA. *Jap Med J*, November, 1959, No. 1885: 13–21. (*Okayama U. School of Med., Japan)

Four patients with tuberculous meningitis are reported whose main symptoms were psychotic disorders. Three patients had previously received

or were under antituberculosis chemotherapy at the onset of their illness, and the usual signs and symptoms of tuberculous meningitis were meager. Initial symptoms of the disease, such as sleeplessness, headache, and malaise, were overlooked or were falsely interpreted as the side effects of chemotherapy in some cases. The main psychiatric manifestations were dementia, delirium (especially at midnight), or convulsions. The symptoms at the onset were usually reminiscent of dementia paralytica progressiva. The spinal fluid was negative for tubercle bacilli in all of the cases. The first case was confirmed at autopsy, and the second case showed hypothalamo-mesencephalic symptoms such as polyphagia, transient glycosuria, obesity, and Korsakow's symptoms during the course. The authors attribute these unusual manifestations of tuberculous meningitis to the incomplete chemotherapy for pulmonary tuberculosis, which could not arrest the spread of the bacilli to the meninges, but suppressed the development of florid manifestations of the disease.—I. TATENO

Hepatic Involvement in Extrapulmonary Tuberculosis. R. KORN*, W. KELLOW, P. HELLER, B. CHOMET, and H. ZIMMERMAN. *Amer J Med*, July, 1959, 27: 60–71. (*U. of Illinois College of Med., Chicago)

Hepatic function was studied in 50 patients with extrapulmonary tuberculosis. Abnormalities of one or more of the liver function– and/or serum protein tests were found in all of the patients. The most frequent changes were impaired bromsulfalein excretion and hyperglobulinemia. Analysis of these results revealed several patterns of hepatic dysfunction which have been described previously with other diseases. These patterns are: (*1*) the elevated serum alkaline phosphatase and bromsulfalein retention associated with space-occupying lesions (granulomas), (*2*) the abnormal flocculation tests and hyperglobulinemia associated with certain chronic infections and inflammatory diseases, and (*3*) combinations of (*1*) and (*2*) which may simulate intrinsic hepatic disease except for the relatively lower levels of serum bilirubin.

Liver biopsy specimens were obtained in 30 of the patients. Granulomas were demonstrated in 24 (80 per cent) of the specimens. Fourteen of the 15 patients with elevated alkaline phosphatase levels had hepatic granulomas. Ten of the 15 patients without alkaline phosphatase elevations also had granulomas. Microgranulomas were described and were considered significant. Focal Kupffer-cell hyperplasia and diffuse sinusoidal inflammatory reaction were sufficiently frequent to be characteristic of hepatic involvement in tuberculosis. Abnormality of the hepatic parenchymal cells was commonly observed, and in 3 patients free hyaline bodies were present (Authors' summary).—T. H. NOEHREN

Fertility and Genital Tuberculosis in Females (in German). P. DORNJAK*. *Wien Med Wschr*, March, 1959, 13: 175–277. (*Zagreb, Petrova ul. 13, Yugoslavia)

Genital tuberculosis was found in 56 sterile women (8 per cent) of 700 examined during a period of 4 years. The diagnosis was made by bacteriologic and histologic examinations of the biopsied endometrium. After treatment with dihydrostreptomycin, isoniazid, and PAS, 3 patients became pregnant, 1 of them twice. Two gravidities terminated in miscarriage, 1 in normal delivery, and 1 pregnancy was ectopic. The end result of pregnancy in women with genital tuberculosis is always moot. It does not imperil the patient if treated with antimicrobials. It is believed that in case of barrenness for about 2 years, every nonhazardous diagnostic procedure for genital tuberculosis should be done.—Z. VIRÁGH

So-Called Primary Ulcerohypertrophic Ileocecal Tuberculosis. F. PAUSTIAN* and H. BOCKUS. *Amer J Med*, September, 1959, 27: 509–518. (*U. of Pennsylvania, Philadelphia)

A brief review is presented of the symptomatology, roentgen features, histopathology, and therapy of hypertrophic ileocecal tuberculosis. A case of ulcerohypertrophic ileocecal tuberculosis without active pulmonary disease is reported. An excellent result followed surgical resection and subsequent antituberculous therapy. The rare occurrence of tuberculous enteritis in the absence of active pulmonary disease is indicated by the fact that only 8 reports have appeared in this country, recording a total of 25 cases since Crohn and Bockus' review of the literature in 1940. Seven were classified as hyperplastic, 6 as ulcerative in type, and 3 were designated as examples of ulcerohyperplastic type. Nine were unclassified. Of this entire group of reported cases, there was only 1 in which the organism was cultured and recovered following guinea pig inoculation from resected tissue, and 2 cases in which the acid-fast bacillus was demonstrated histologically.—T. H. NOEHREN

Tuberculosis Originally Diagnosed as Retothelial Sarcoma (in German). H. JELLINEK*. *Wien Med Wschr*, April 25, 1959, 17: 358–359. (*Gmunden, Kammerhofgasse 6, Oberosterreich)

An ulcer of the glans penis was treated first with roentgen therapy and later with radium

irradiation after the biopsied specimen was diagnosed as possible retothelial sarcoma. After 5 years of treatment no improvement was noted, and the patient changed his physician. On the basis of family history and a pulmonary roentgenographic finding of old pleural changes, chemotherapy with the 3 major conventional antimicrobials was instituted and resulted in complete healing.—Z. VIRÁGH

Treatment of Tuberculosis of the Spine. M. C. WILKINSON*. *Brit Med J*, August 29, 1959, No. 5147: 280–282. (*Black Notley Hosp., Essex, England)

Between the years 1948 and 1958, 138 operations, usually preceded by antimicrobial therapy for 14 to 18 weeks, were performed on 128 patients with spinal tuberculosis. There was no operative mortality. Bony ankylosis was obtained in 82, stable fibrous ankylosis in 40, and unstable fibrous ankylosis in 8. The most important operative complication was tension pneumothorax, which occurred twice. The majority of patients had lesions involving the thoracic and thoracolumbar spine, and the results compared favorably with 105 patients with disease of the lumbar area who did well on medical treatment alone. Twenty-one patients were later readmitted for reactivation of their disease. The over-all mortality rate was less than 2 per cent. The average duration of treatment was 9.7 months.—E. A. RILEY

UPPER RESPIRATORY TRACT DISEASE, VIRAL

***Schweiz Med Wschr*, August 22, 1959, 89: pp. 877–888** (in German):

Adenovirus Infections Type 4 and 7a in Eastern Switzerland in 1958: Clinical, Virologic, and Serologic Studies. G. KAUFMANN*, T. WEGMANN, M. RENTSCH, and E. WIESMANN: pp. 877–882. (*Kantonsspital, St. Gallen, Switzerland)

From January to September, 1958, 92 adenovirus strains were isolated from 61 patients. Most of them were found between April and July. A more detailed report is given of 32 cases which were examined serologically. In 26 of these cases, virus was isolated. In 15 cases, at least a fourfold increase in the titer was recorded between the determinations in the early and in the late phases of the diseases. In each of the remaining cases an agent with typical cytopathogenic effect was isolated, and the reconvalescent serum gave complement-fixation titers of 1:80 or more, but no serum was available in the acute phase. Ten patients had a fourfold or better rise in neutralizing antibody titer for types 4 or 7a.

The 15 civilian patients presented respiratory illness, which was associated with pulmonary consolidation in 3 cases. The clinical picture corresponded roughly to pharyngoconjunctival fever with frequent meningism. Cerebrospinal fluid was normal in all instances. The 17 military patients presented respiratory illness which was associated with pulmonary consolidation in 6 cases; 15 cases occurred in the same recruit training unit. The connection between the civilian and military epidemics is suggested by a few contact cases.

The rise in the complement-fixation titer occurred not earlier than 6 days after the onset of the disease. Significant titer rises occurred as late as between the second and third weeks of illness. Virus excretion in the feces was observed up to 3 months after the onset.

Lung Infiltrations during Adenovirus Infections. T. WEGMANN*, G. KAUFMANN, and E. WIESMANN: pp. 882–887. (*Kantonsspital, St. Gallen, Switzerland)

Nine cases are reported of lung infiltrations associated with adenoviral infection. The courses were febrile or afebrile. Auscultatory findings suggested pneumonia or bronchitis, but some cases were asymptomatic on auscultation. The roentgenographic changes consisted of mottling and peribronchial infiltration or homogenous density. The hilar shadows were always enlarged. The other clinical symptoms corresponded to adenoviral infection without pulmonary consolidation. Some cases showed transient positive complement-fixation titers with ornithosis antigen, and transient positive syphilis reactions. These were regarded as etiologically nonspecific or "false" positive reactions.

Specific Laboratory Diagnosis of Adenovirus Infections. E. WIESMANN*: pp. 887–888. (*Bakteriologisches Institut, St. Gallen, Switzerland)

A review is presented of the laboratory methods to be used when adenoviral infection is suspected: isolation of virus and serologic examinations. —J. HAAPANEN

The Asiatic Influenza in Finland, 1957. K. OHELA* and W. J. KAIPAINEN. *Ann Med Intern Fenn*, No. 3, 1959, 48: 111–125. (*U. of Helsinki, Finland)

According to official statistics, from the beginning of the influenza epidemic in September, 1957, 626,984 persons of Finland's total population of 4,315,000 contracted the disease. Although the disease was generally mild, its complications, e.g., pneumonia, were serious. The mortality rate

was 210.8 per 100,000 diseased. Mortality was especially high in the population group older than 65. Compared with earlier influenza epidemics, the 1957 epidemic was bigger as regards morbidity, but the mortality rate in the 1953 epidemic was higher.—E. DUNNER

THORACIC SURGERY

Cardiac Arrest Outside the Operating Room with Successful Resuscitation: Report of Two Cases. C. MOTA*, E. ZIKRIA, and R. TABER. *Ann Intern Med*, September, 1959, 51: 577–583. (*Henry Ford Hosp., Detroit, Mich.)

A review of the literature concerning successful resuscitation in cardiac arrest occurring outside the operating room reveals 13 cases. Two additional patients who have made complete recoveries are reported. In the future there should be more instances of successful resuscitation of patients dying suddenly from coronary artery disease. A patient who has a recent history of a mild coronary attack and is in fairly good general condition, or one who has been in apparently good health prior to the sudden arrest, would logically be a candidate. Resuscitative measures of this type should not be used in the merciful death of patients in the terminal stages of cancer, stroke, or congestive heart failure.
—T. H. NOEHREN

Pulmonary Distomiasis Successfully Treated by Lobectomy (in Japanese). T. IWAMA*, T. NAKANO, and S. TADA. *Clin Radiol*, December, 1959, 4: 916–919. (*Fujimi Hosp., Shizuoka Prefecture, Japan)

Pulmonary distomiasis is endemic in about 6 of 46 prefectures in Japan; the writers report the case of a 21-year-old male whose chief complaint was intermittent hemoptysis of 9 months' duration. Because of the refractoriness of the hemoptysis to a course of emetine hydrochloride (400 mg.) and arsenicals, the patient underwent left lower lobectomy. Preoperatively, his leukocyte count was 5,200 per cu. mm. with 10 per cent eosinophils, and his sputum was repeatedly positive for distoma ova. A cavity 10 mm. x 10 mm. in size was found in the lung, which was encapsulated and contained yellowish pus, a distoma worm, and many ova. The postoperative course was uneventful. The writers recommend surgical treatment of this disease, as emetine treatment is liable to relapse and no standard method of internal therapy is available at present. Four other papers are mentioned dealing with successful treatment of this disease by surgery.—I. TATENO

Spontaneous Perforation of the Esophagus: Review of the Literature and Report of a Case. E. MARSTON* and H. VALK. *Ann Intern Med*, September, 1959, 51: 590–607. (*Bowman Gray School of Med., Winston-Salem, N. C.)

A case is presented of perforation of the esophagus possibly secondary to vomiting. The history of this disease and its signs, symptoms, and diagnosis are discussed. The various etiologic factors are presented. Certain local physiopathologic conditions which may play a part in determining the perforation are discussed. From the available literature, a total of 232 patients with this condition has been collected, 119 of whom underwent some surgical procedure. The over-all surgical mortality was 36 per cent. Only by surgical attack can these patients be saved (Authors' summary).
—T. H. NOEHREN

Traumatic Diaphragmatic Hernia. E. LAUSTELA* and P. TALA. *Ann Chir Gynaec Fenn*, Supp. 87, 1959, 48: 1–45. (*U. of Helsinki, Finland)

From 1942 to 1958, 23 cases of traumatic diaphragmatic hernia were treated by surgery. In 12 cases the hernia was secondary to a war injury, in 4 cases it was caused by a penetrating wound, in 3 cases by an automobile accident, in 2 by hyperemesis, in 1 by a blow to the abdomen, and in 1 by falling from a height of 10 meters. In 2 instances the hernia was on the right, in 21 on the left. In the cases of right-sided hernia, the liver, but no other abdominal organs, was herniated into the thoracic cavity. On the left, the most commonly dislocated organs were the omentum, colon, stomach, and the small intestine. In 4 cases the spleen had penetrated above the diaphragm, and in 2 cases part of the pancreas. The patients frequently also had other lesions secondary to the same trauma, such as pleural empyema, intrapulmonary or abdominal foreign body, concussion, bone fractures, nerve lesions, et cetera. Gastrointestinal symptoms were the most predominant: pain and vomiting, swelling, and meteorism. Dyspnea was present in 5 patients, and chest pain was the sole symptom in 2 cases. Laparotomy was performed on an emergency basis on 3 patients because of symptoms of strangulation. No intestinal or ventricular resection was necessary.

For all 23 patients, correction of the diaphragmatic hernia was performed by thoracotomy. In 19 cases the defect was closed by suturation of the margins of the laceration. In 4 cases myoplasty had to be performed. One of the wartime patients died of pneumonia and paralytic ileus on the third postoperative day, and another patient died 2 years after the operation, possibly

of strangulated recurrent hernia. The late results of the surgical treatment can be considered good in 6 and satisfactory in 3 of the wartime patients. Nine of the others had resumed their work and their general condition was good. Two patients had slight abdominal and thoracic symptoms. —J. HAAPANEN

The Concept of Pericardiectomy as a Treatment for Recurrent Idiopathic Pericarditis in the Absence of Cardiac Constriction. H. F. ZINSSER*, J. JOHNSON, and W. S. BLAKEMORE. *Amer J Med Sci*, October, 1959, 238: 464–470. (*Hosp. of U. of Pennsylvania, Philadelphia)

Pericarditis can produce a form of disability entirely separate from pericardial constriction or tamponade. Six patients with idiopathic pericarditis had a smoldering, febrile illness characterized by recurrent severe chest pain, with constitutional symptoms and pleuritis. These patients failed to respond to standard treatment and developed long-term disability with multiple hospitalizations, inability to work, and a state approaching total invalidism. Pericardiectomy, performed primarily to interrupt this process rather than to treat or prevent pericardial constriction or tamponade, restored all 6 patients to complete health.—W. J. STEININGER

Complete Aplasia of the Pericardium (in Japanese). H. TAKAKURA*, K. TOYODA, K. NISHIHARA, and T. SUZUKI. *Jap J Thoracic Surg*, November, 1959, 12: 1050–1053. (*National Hyogo Sanatorium, Japan)

A case report is presented of a 26-year-old male patient whose pericardium was unexpectedly found to be absent when he underwent pulmonary resection for tuberculosis. This is the second such case reported in Japan. The patient's past history was noncontributory except that he had dyspnea on hard physical exercise. Postoperatively, he had dyspnea and cough, re-expansion of the lung was poor, and the electrocardiographic findings were very variable when his physical position was changed, indicating a very unstable heart. His lung re-expanded, dyspnea and cyanosis were absent, and other physical and laboratory examinations returned to the normal preoperative levels two months after operation.—I. TATENO

Carcinogenic Properties of Various Plombage Materials (in German). W. SCHULTZE-BRÜGGEMANN*. *Beitr Klin Tuberk*, March, 1959, 120: 18–33. (*Sanatorium "Schildautal," Seesen a. Harz, Lautenthaler Strasse, Germany)

Plexiglas, paraffin, perlon, and polystan employed in pulmonary plombage were investigated in surgically removed specimens and in those obtained by autopsy. In no case was evidence elicited that any one of these materials had carcinogenic properties. In the literature, there is not a single case reported in which the development of pulmonary malignancy might have been connected with the carcinogenic effect of polymers.—Z. VIRÁGH

Results of Pulmonary Function Studies in Partial Pulmonary Resections with Complementary Osteoplastic Thoracoplasty (in French). P. DAUMET*, Y. DELANOE, and F. BURGINE. *Rev Tuberc (Par)*, August–September, 1959, 23: 834–844. (*Paris, France)

The results of pulmonary function studies are compared in cases of lobectomies performed with and without Bjork's method of thoracoplasty. The results are identical regarding vital capacity, maximal breathing capacity, and the relation of maximal expiratory volume per second to vital capacity (VEMS/VC). The authors conclude that this method of thoracoplasty is perfectly justified from the functional point of view. Even in cases in which the indication is not absolute, it does not increase respiratory deficiency. In cases in which it is possible to perform lobectomy plus segmental resection instead of pneumonectomy, the remaining parenchyma retains a satisfactory functional capacity.—V. LEITES

Surgical Treatment of Patients with Pulmonary Tuberculosis Complicated by Diabetes (in Japanese). C. SUZUKI*, T. KONDO, and K. HASHIMOTO. *Jap J Clin Tuberc*, November, 1959, 18: 750–762. (*Tohoku U., Sendai, Japan)

An analysis of the results of surgical treatment in 27 cases (10 thoracoplasty procedures and 17 pneumonectomies) and a review of the literature are presented. Most of these patients developed diabetes during treatment for pulmonary tuberculosis, and half of them were more than 40 years of age. Many cases were asymptomatic or mild; few cases were severe. Diabetes could be controlled adequately by diet and insulin therapy before operation in most cases, and systematic antituberculosis chemotherapy and anesthesia could be given and surgery performed, as in the uncomplicated cases, with a few exceptions.

The following points were stressed for the treatment of these patients: oatmeal and insulin on the morning of surgery; sufficient oxygen, irrespective of the the type of anesthesia, to prevent the development of anoxia; measurement of the blood sugar level every 30 minutes during, and every 2–4 hours after operation; insulin and 5 to 10 per cent glucose solution if indicated; a high carbohydrate–high-fat meal when the patient recovers from anesthesia; control of the diabetic and metabolic states, especially during the

first 1–2 weeks postoperatively; and, sufficient chemotherapy. When these precautions are carefully taken and controlled, the results of treatment may be as favorable as in patients without the diabetic complication.—I. TATENO

ROENTGENOGRAPHY

Roentgenologic Considerations of Capillary Bronchiolitis. D. KOCH*. *Amer J Roentgenol*, September, 1959, 82: 433–436. (*Port Huron, Mich.)

Capillary bronchiolitis can be described as an acute clinical syndrome characterized by respiratory distress and emphysema in an acute respiratory infection in infants. The fact that it is an acute condition and that it is found in infants distinguishes it from lung changes in cystic fibrosis of the pancreas and emphysema due to ordinary allergy. Similarly, the lung changes can be readily differentiated from overinflation of the chest due to exposure of the film during maximal inspiration.

In spite of the fact that the prognosis generally is very favorable and the pathologic changes are few, certain features appear to be characteristic. Grossly, the lungs are strikingly emphysematous. There are scattered areas of atelectasis and consolidation. The air passages from the pharynx to the bronchi show hyperemia. No obstructions are noted in the trachea or major bronchi. Microscopic examination reveals a severe thickening of the walls of the small bronchi and bronchioles as a result of lymphocytic infiltration. Mild inflammatory exudate extends into the adjoining alveolar septa and moderate infiltrates of neutrophils are found in bronchiolar lumina and alveolar spaces. There is severe emphysema present in addition to the patchy areas of atelectasis and bronchopneumonia.

Often the roentgenologist is confronted with a frontal roentgenogram of the chest in which the lung fields appear unusually clear and the diaphragms are pulled down at the height of inspiration. The impression is one of optimal technical detail and exposure at the most perfect time in the respiratory cycle. Paradoxically, this so-called "too normal"-appearing chest roentgenogram is found to be that of an acutely and desperately ill infant. Roentgenograms in inspiration and expiration in both frontal and lateral views give the most information and have been adopted as routine. On the frontal roentgenogram in inspiration, the lungs show increased radiolucency with depressed diaphragms where the variations in the crura produce a scalloped effect. The costophrenic sulci are broadened and the acuity of the angles is obliterated. The vascular shadows appear to be stretched out, particularly from the hili to the median bases in the overdistended lung. On the frontal roentgenogram in expiration, the obstructed portions of the lungs become more apparent as the unobstructed parts lose their radiolucency. Atelectatic areas and segments of consolidation become more obvious. Finally, the over-distended lung apparently exerts enough pressure on the heart to reduce its size, whereas in the normal chest the heart shadow increases in size during expiration. This paradoxical change in heart size is probably a sign of an added burden on the already overworked heart.

On the lateral roentgenogram of the chest, perhaps the earliest evidence of lung abnormality is the depressed surface of the diaphragm in the posterior halves of the chest. This is often a reversal of the normal convexity.—T. H. NOEHREN

Segmental Bronchography Performed Under Pressure (in Russian). N. D. SCHIFMAN*. *Probl Tuberk*, No. 7, 1959, 37–40. (*Institute of Tuberculosis, AMN, U.S.S.R.)

Segmental bronchography was performed in 120 patients. Local topical anesthesia was used. The catheter was introduced into the segmental bronchus under fluoroscopic visualization. The contrast medium was injected with a syringe under pressure and roentgenograms taken. Segment after segment can thus be filled and roentgenographic records made.

Three illustrative cases are presented. In a patient who had undergone therapeutic pneumothorax for 2 years without benefit, a segmental bronchogram showed a cavity in the posterior segment and it was resected. A post-thoracoplasty patient had persistently positive sputum for which tomography failed to show the source. Segmental bronchography under pressure revealed a cavity in the posterior segment of the right upper lobe. The third patient began to cough up large amounts of sputum 3 years after resection of the right lower lobe. Roentgenograms and tomograms failed to show the source of the copious sputum. Ordinary bronchography showed a normal bronchial tree in the upper and middle lobes. Bronchography under pressure revealed a fistula between the stump of the lower lobe bronchus and an empyema pocket. No complications were observed in any of the patients who had segmental bronchography.—W. A. ZAVOD

The Application and Advantages of Cobalt-60 Supervoltage in the Radiation Therapy of Cancer. J. LEHMAN*. *Mississippi V Med J*, November, 1959, 81: 283–285. (*Jackson Heights, N. Y.)

Cobalt 60 does not exist in nature; it is produced in a reactor and requires 18 months of neutron irradiation before its specific activity is high enough to be used for therapy. Cobalt 60 emits only gamma rays, uniform and of a very high energy radiation and, thus, penetrative ability.

The isotope has a half-life of 5.3 years. The author's unit, comparable to a 3 million-volt X-ray machine, contains 1,000 curies—more than could be obtained by pooling all of the radium in the world. Its advantages are: (*1*) increased skin tolerance (4,000–5,000 r produce only a faint erythema); (*2*) dependability (no electrical problems related to fluctuation of line voltage, no oil and water cooling systems); (*3*) predictable output (isodose curves resemble those of a 3 million-volt X-ray machine); (*4*) increased depth dose with more rapid courses of treatment; (*5*) less side scatter (with less radiation sickness and blood and bone marrow effects); (*6*) no soft energy filtration needed; (*7*) decreased bone and cartilage absorption, reducing the danger of necrosis.—E. ROTHSTEIN

Accelerated Palliative Radiation Therapy of Bronchial Carcinoma with 250-kv Roentgen Rays. W. L. DEGINDER* and B. K. LOVELL. *Radiology*, November, 1959, 73: 684–692. (*2410 Rio Grande, Austin, Texas)

An accelerated radiation technique employing a tumor dose of 1,500 to 3,200 r in 8 days or 2 weeks was used in a series of patients with nonresectable bronchial carcinoma. The rate of survival in this group, calculated from the start of radiation therapy, compares favorably with that in a similar group treated by a conventional plan (2,000 to 6,000 r in 3 to 6 weeks elapsed time). Alleviation of symptoms was obtained without prolonged hospitalization or unpleasant sequelae. Quite as important was the relief of anxiety and creation of hope among these patients.—W. J. STEININGER

Treatment of Bronchogenic Cancer with Conventional X-Rays According to a Specific Time-Dose Pattern. S. RUBENFELD* and G. KAPLAN. *Radiology*, November, 1959, 73: 671–678. (*New York U. College of Med., N. Y.)

Forty-three patients with advanced nonresectable cancer of the lung were treated with a specific time-dose pattern of 3,000 r in 2 weeks. Symptoms were controlled in 72 per cent of the series. Objective improvement, mainly regression as noted roentgenographically, was observed in 40 per cent of the patients. Six patients are surviving from 2 to 28 months. The average survival period for all of the patients at the time of this survey was 5.1 months. An abbreviated time-dose pattern achieved satisfactory palliation, shortened the hospitalization period, and did not induce serious debilitation. There was no need to interrupt treatments because of radiation sickness, tracheitis, esophagitis, or skin reaction (Authors' summary).—W. J. STEININGER

Carcinoma of the Esophagus: Cobalt 60 Teletherapy. M. F. MAGALOTTI* and I. F. HUMMON. *Mississippi V Med J*, November, 1959, 81: 263–266. (*Cook County Hosp., Chicago, Ill.)

Carcinoma of the esophagus is radiosensitive and "radiocurable." This, the most common serious esophageal disease, is most frequent in old men; it is typically epidermoid and progresses rapidly through the wall of the esophagus into adjacent structures, invading trachea, aorta, and other major vessels, and the lymphatics and bronchi. These factors render surgical treatment far from satisfactory. The earlier cobalt 60 radiotherapy is begun, the better the results. The beam is equivalent to a 3 million-volt X-ray machine, producing a cancerocidal dose of excellent penetration—6,000 r. Forty patients were treated only by cobalt 60 therapy—an average of 30 treatments, about 10 minutes each, over a 6-week period, many on an out-patient basis. Five are alive and well, the longest survival being 4 years. Three died of unrelated disease 1–2 years after treatment, and no tumor was found at autopsy. Seventy-eight other patients were treated with cobalt 60 after surgery or "palliatively." All died within a relatively short time.—E. ROTHSTEIN

Treatment of Pleural and Peritoneal Effusion with Intracavitary Colloidal Radiogold (Au^{198}). J. DYBICKI, O. J. BALCHUM, and G. R. MENEELY*. *AMA Arch Intern Med*, November, 1959, 104: 802–815. (*Vanderbilt U. Hosp., Nashville, Tenn.)

Intracavitary radioactive colloidal gold (Au^{198}) was administered to patients with pleural and peritoneal effusions due to local or metastatic malignancy. Employing intrapleural dosage of 125 millicuries (mc.) and intra-abdominal dosage of 150 mc. of Au^{198}, good results were obtained in 12 of 22 patients (54.5 per cent) with pleural effusions, and 10 of 17 patients (58.8 per cent) with peritoneal effusions. Administration of radiogold is accompanied by few serious side effects and has no real hazards to patient or personnel when used properly. Although only palliative and not curative, intracavitary Au^{198} can successfully relieve the distressing consequences of protein and electrolyte loss in patients with malignant effusions, and can result in distinct symptomatic relief.—W. J. STEININGER

The Diagnosis of Obstructive Pulmonary Emphysema in Mass Radiography. K. A. J. JÄRVINEN* and K. THOMANDER. *Ann Med Intern Fenn*, No. 3, 1959, 48: 151–156. (*U. of Helsinki, Finland)

An endeavor was made to ascertain by mass roentgenography the incidence of obstructive

pulmonary emphysema (OPE) in a series of 10,760 able-bodied persons. The disease was suspected roentgenographically (single postero-anterior film only) in a total of 159 subjects; 100 of them were given a thorough clinical examination including pulmonary function tests. The diagnosis of OPE was confirmed in 42 per cent of the cases. Restrictive pulmonary disease was established in 8 per cent of the patients. Fifty per cent of the suspects had healthy lungs. The majority of the subjects with healthy lungs, in whom OPE had been suspected from the mass survey films, were elderly males. The most important reasons for the mistake were believed to be the pulmonary ("senile pulmonary emphysema") and thoracic changes (kyphosis, et cetera) thought to be physiologic and associated with more advanced age. Considering the results and the extensive use of mass roentgenography, the method can be considered suitable for a preliminary screening examination to detect OPE in a major population group. Diagnosing individual cases of OPE, however, always requires clinical examination.—E. DUNNER

Radiation Therapy in the Management of Neoplastic Obstruction of the Superior Vena Cava. R. M. JAMISON*, T. E. KEATS, E. M. STEVENS, and P. C. WHEELER. *Mississippi V Med J*, November, 1959, 81: 273–278. (*U. of Missouri Med. Center, Columbia)

The authors use supervoltage radiotherapy (a 2 million electron-volt Van de Graaff generator) which results in greater penetration with higher dose levels in a short period of time. A dose of 3,000–5,000 r is delivered (200 r daily tumor dose) in 15–20 days. An occasional slight increase in symptoms may be noted at first, but marked, if temporary, improvement is the rule. A number of illustrative cases are presented, with roentgenograms.—E. ROTHSTEIN

Modern Roentgenological Methods in the Detection and Analysis of Small Pulmonary Calcifications. I. LINDGREN*, P. SOILA, and C. LAGERGREN. *Acta Path Microbiol Scand*, No. 4, 1959, 47: 342–352. (*U. of Turku, Finland)

Detection of small pulmonary calcifications was carried out by macroscopic roentgenography of air-filled trachea–lung specimens on nonscreen film. The exposure factors were 50 kVp (4-valve circuit), 150 mA, and 0.32 sec. at a distance of 150 cm. Short exposure time was preferred because the air-filled lung tended to move during exposure. Further analysis was performed of slices of lung tissue roentgenographed on dental film with 35 kVp, 120 mA, 0.8 sec. at a distance of 100 cm. The smallest calcifications found were 0.3 mm. in length. Conventional staining methods and microroentgenography were carried out on consecutive specimens. Powder diffraction and microdiffraction analyses showed that the inorganic component of the calcifications consisted of hydroxy apatite containing excessive amounts of CO_2. This may be explained by the ion-binding capacity of apatite and its location in the CO_2-rich milieu of the lungs.—E. DUNNER

A Radiological Analysis of Disseminated Lung Disease. D. M. GOULD* and G. V. DALRYMPLE. *Amer J Med Sci*, November, 1959, 238: 621–637. (*U. of Colorado Med. Center, Denver)

In the problem of differential diagnosis of disseminated lung disease, the attending physician and the roentgenologist can help each other by pooling information, stimulating particular lines of inquiry, and by final critical analysis of all of the available data. The roentgenologist frequently can help by a thorough analysis of the roentgen pattern according to this outline: (*1*) description of the lesion: size, shape, number, and density; (*2*) anatomic localization: bronchoalveolar, vascular, and interstitial; (*3*) pathologic process: inflammatory and neoplastic; (*4*) pathogenesis and route of dissemination: bronchoalveolar, vascular, and interstitial; (*5*) differential diagnosis; and (*6*) recommendations for further study. Five comprehensive tables of disseminated lung disease are presented listing etiology by alphabetic order and by route of dissemination, anatomic location, morphologic changes in the circulatory system, and miscellaneous undiagnosed lung diseases.—W. J. STEININGER

The Vascular Pattern of the Lung as Seen on Routine and Tomographic Studies. E. MICHELSON* and J. O. SALIK. *Radiology*, October, 1959, 73: 511–526. (*Sinai Hosp. of Baltimore, Md.)

After some experience with tomographic localization of pulmonary structures, most of the vascular pattern can be identified with surprising ease on routine postero-anterior and lateral films. This knowledge is valuable in appreciating the great spectrum of pathologic changes, congenital or acquired. The pulmonary arteries arch downward from the hilus, producing a weeping-willow pattern. The pulmonary veins enter the mediastinum below the arteries and differ in their course from the artery in describing an arc upward and outward rather than downward and outward and, in so doing, cross the artery and bronchus somewhere within the middle portion of the lung field. The veins produce a very characteristic design, being straighter than the arteries with branches at less acute angles. In the segmental plane the

vein is either lateral or inferior to its corresponding artery. Since the vein and artery cross at right angles, the differentiation can be made by following the course of the vessel back toward the hilus. Illustrative roentgenograms are presented, together with 3 two-color schematic diagrams.—W. J. STEININGER

Pulmonary Radiation Reaction: A Vital-Capacity and Time-Dose Study. J. R. GISH*, E. O. COATES, L. A. DUSAULT, and H. P. DOUB. *Radiology*, November, 1959, 73: 679–683. (*Henry Ford Hosp., Detroit, Mich.)

Study was made of the pulmonary radiation reaction with reference to the time-dose relationship in 134 patients (the majority being treated for carcinoma of the breast) in whom the lungs received incident radiation during therapy to the mediastinum, lungs, and node-bearing areas of the chest walls. In addition, 45 cases of pulmonary fibrosis, in which time-dosage figures were available, were taken from the literature. The maximal dose to the lung varied from 2,000 r in 5 days to 6,000 r in 82 days in the cases treated at the Henry Ford Hospital, and from 1,700 r in 12 days to 8,200 r in 30 days in those collected from the literature. Forty-four per cent of the patients who received 250-kv roentgen therapy showed some evidence of pleuropulmonary reaction with some degree of permanence roentgenographically. In those receiving cobalt-60 therapy, only 20 per cent showed pleuropulmonary reaction despite higher tissue dose. The small degree of lung reaction is largely attributable to tangential therapy and modern methods of lung-shielding in routine cases. Among patients who showed pulmonary reaction, vital capacity studies showed mild changes from the normal. None of these patients exhibited clinical signs of impaired pulmonary function.—W. J. STEININGER

Concern About Radiation in X-Ray Diagnosis . . . Real or Unreal. H. V. VON GAL*. *Arch Pediat*, November, 1959, 76: 437–446. (*Greenwich Hosp., Connecticut)

The problem of minimizing radiation exposure as it relates to pediatrics in roentgenographic diagnosis is not one of discontinuing necessary informative examinations, but concerns the judicious use of proper equipment. Routine fluoroscopic evaluation of infants and children should be discontinued. Fluoroscopic evaluation of an infant done with out-dated equipment and large fluoroscopic fields may cause the gonads to receive an amount of X-ray exposure equal to many thousand properly taken chest films. All X-ray equipment should be routinely calibrated by a radiation physicist, and his recommendations followed. The more common examinations, such as chest, skull, and extremities, give negligible amounts of radiation exposure to the gonads. Examinations around the pelvic area, which naturally give higher gonadal exposures, should only be done when specifically indicated and then with the proper equipment and precautions. If this is followed, there should be no real concern about radiation exposure in any diagnostic roentgen procedure.—M. J. SMALL

The Bronchographic Picture in Silicotuberculosis (in Italian). A. FERRARIS* and G. PAOLI. *Lotta Tuberc*, July–August, 1959, 29: 1031–1050. (*Clin. Tisiologia, U. of Genoa, Italy)

The bronchographic results obtained in 27 patients with silicotuberculosis are briefly given for each case, and it is derived from them that a bronchographic picture in silicotuberculosis is drawn with 11 special patterns. It is concluded that no one particular sign exclusively characteristic of the disease exists; the association of the bronchographic aspects of silicosis with those of tuberculosis can, however, be a real aid in correct diagnosis.—I. ARCHETTI

The Roentgenographic Lung Image in Mitral Stenosis (in Spanish). F. GALLAND*, S. ARANDA, S. ACEVES, and N. DORBECKER. *Rev Mex Tuberc*, March–April, 1959, 20: 101–119. (*National Institute of Cardiology, Mexico, D. F.)

A series of 20 cases of rheumatic heart disease with pure or predominant mitral stenosis was studied. All cases had a mitral valve area of less than 1 cm. as determined by the surgeon at the time of commissurotomy. The patients ranged in age from 13 to 45 years; 10 were males and 10 females. Studies included postero-anterior and right and left anterior oblique roentgenograms, both pre- and postoperatively, as well as clinical evaluation and preoperative hemodynamic studies. The preoperative roentgenograms were taken in most instances within a few days to 2 months of the catheterization studies. The postoperative roentgenograms were taken within 2 months after surgery, and then between 2 and 6 months, between 6 months and 1 year, and after 1 year of surgery.

For the purposes of this study, pulmonary capillary hypertension was graded as follows: Grade I—12 to 15 mm. Hg, II—16 to 20 mm. Hg, III—21 to 30 mm. Hg, and IV—over 30 mm. Hg. Clinically, the cases were classified as follows: Grade I—dyspnea on moderate to severe exertion, II—dyspnea on slight exertion, III—dyspnea decubitus or orthopnea, IV—paroxysmal dyspnea,

hemoptysis, or bloody expectoration. The mitral valve area was classified as follows: Grade I—1.6 to 2.6 cm.², II—1.1 to 1.5 cm.², III—0.6 to 1 cm.², IV—0.5 cm.² or less. The pulmonary roentgenographic images were classified as follows: Grade I—hilar haziness; II—more mottled, fine, and diffuse hilar haziness; III—hilar haziness plus linear streaking (with or without fine mottling); IV—hilar haziness plus abnormal horizontal fissure or Kerley's B lines (septal lymphatic lines), or both (with or without linear shadows and fine mottling).

The degree of opening of the mitral valve achieved by surgery was classified as follows: Grade IV—when both commissures were opened completely; III—when one commissure was opened completely and the other one partially; II—when both commissures were opened partially, or one completely and the other not opened; I—when one commissure was opened partially and the other unopened; 0—when no commissurotomy was performed. Roentgenographically, preoperatively, all cases showed hilar haziness of various degrees, 14 cases showed linear streaking, fine and diffuse mottling was present in 15, and Kerley's B lines in 17. In all of the cases with Kerley's B lines, the capillary pressure was more than 20 mm. Hg. Postoperatively, the roentgenographic changes which occurred were as follows: (*1*) within 2 months after commissurotomy, the horizontal fissure and the lymphatic septal lines disappeared in all cases; (*2*) within 2 to 6 months, the linear streaking disappeared in all cases; (*3*) after 6 months, the fine mottling persisted in 50 per cent of the cases. The hilar haziness persisted in 66 per cent of the cases, but both of these changes diminished in intensity. There were cases in which the linear streaking, the fine mottling, and the hilar haziness all disappeared within 2 months.

The following preoperative positive correlations were noted: (*1*) between the roentgenographic image and the pulmonary capillary pressure, in 9 cases of 14 studied (64.2 per cent); (*2*) between the clinical picture and the roentgenographic image, in 16 cases of 20 (80 per cent); (*3*) between the roentgenographic image and mitral valve size, in 18 of 19 cases (94.7 per cent); (*4*) between the roentgenographic image and the clinical picture of venocapillary hypertension, in 16 of 18 cases (88.8 per cent); (*5*) between the roentgenographic image and the degree of mitral valve opening achieved by the surgeon, in 17 of 18 cases (94.4 per cent).

The following generalizations are derived from this study: (*1*) Severe mitral stenosis is incompatible with a normal roentgenographic pulmonary image. (*2*) Generally, there is a good positive correlation between the pulmonary roentgenographic shadow in the patient with mitral stenosis, the mitral valve area, and pulmonary venocapillary hypertension. (*3*) The changes which may occur in the pulmonary roentgenographic image after mitral commissurotomy may be used, in general, to judge the effectiveness of the operation.—F. Perez Pina

Tomobronchography in Phthisiology (in French). P. Chadourne*, L. Duchet-Suchaux, J. Ioannou, and A. Pinelli. *Schweiz Z Tuberk*, No. 1, 1959, 16: 4–9. (*Sanatorium de Chevilly, Chevilly-Larue/Seine, France)

This procedure is an important supplement to common bronchography, especially when precise determination of segmental or subsegmental bronchi is required presurgically.—Z. Virágh

CHEMOTHERAPY

Experimental Studies on the Antituberculous Activity of Alpha-Ethyl-Thioisonicotinamide (in Japanese). T. Toda*, T. Tokunaga, Y. Hagiwara, and K. Hisatsune. *Jap J Clin Tuberc*, December, 1959, 18: 862–865. (*Kyushu U., Fukuoka, Japan)

Alpha-ethyl-thioisonicotinamide (Libermann, D., *et al.*, 1957) inhibited the growth of tubercle bacilli at 1 γ per ml. *in vitro*. It was effective against tubercle bacilli resistant to streptomycin, PAS, or isoniazid. Its LD_{50} for mice was 963 mg. per kg. of body weight. It was more effective than streptomycin in the experimental tuberculosis of mice in a daily dose of 1 mg. per animal. It was less effective than streptomycin (10 mg. per day subcutaneously) in the experimental infection of guinea pigs when the daily dose was 10 mg. orally, but it was considerably effective when compared with untreated control animals. The above experiments gave the writers the impression that this compound is a promising antituberculous drug.—I. Tateno

The Role of Cycloserine (Seromycin) Blood Level in the Treatment of Pulmonary Tuberculosis and the Prevention and Control of Cycloserine (Seromycin) Toxicity. C. X. Holmes*, G. E. Martin, and K. I. Fetterhoff. *Dis Chest*, December, 1959, 36: 591–593. (*Pennsylvania Dept. of Pub. Health, Pittsburgh)

The cycloserine serum level was used as the control in a study of 60 treatment-failure cases of pulmonary tuberculosis. It was determined that those cases which maintained a serum level between 20 and 40 γ per ml. of serum showed both roentgenographic and bacteriologic response uncomplicated by cycloserine toxicity. For the first

3 months, cycloserine alone was given; it was combined with isoniazid for the remaining 6 months of the study. There was sputum conversion in 25 per cent and regression of roentgenographic lesions in 42 per cent of the cases studied. —E. A. Rouff

Chemotherapy in the Prevention of Relapse in Patients Who "Require Careful Observation" (in Japanese). H. Okada*, O. Kojima, T. Kato, K. Kuriyama, I. Oya, and N. Miyashita. *Jap J Clin Tuberc*, November, 1959, 18: 780–784. (*Nagoya U. School of Med., Japan)

Patients with minimal tuberculosis that does not require active and vigorous chemotherapy but calls for careful observation, or patients who have undergone various types of treatment in the past and are not under present treatment, are usually seen among the employees of a large company or factory. Recrudescence or activation of the disease in these persons are important problems from clinical and public health standpoints.

The present study was made on 202 such patients from 2,400 employees of a company. Daily Na methansulfonate of isoniazid (Neoiscotin), 0.6 gm., was given for 6 to 24 months to the study group, and the others were not treated. Significantly more untreated cases of the CB type (mainly fibrocaseous and partly infiltrative-caseous pulmonary lesions) became worse. No significant difference was seen in cases of the CC type (mostly fibrocaseous lesions), and in 30 per cent of the treated cases the lesions became less pronounced. All of the cases with D-type lesions (sclerotic) were untreated, and the results were the same as in the CC group.

When surgically treated patients were treated for more than 6 months after return to work, there were no relapses. However, when they were left untreated, 14.3 per cent of the patients who had undergone thoracoplasty relapsed within 2 to 8 years, and 12.5 per cent of those who had pulmonary resection relapsed within 4 years. In conclusion, the CB cases should be actively treated, and the surgically treated patients should receive chemotherapy for more than 6 months after they return to work.—I. Tateno

Bacterial Drug Resistance at the Time of the Patient's Admission to Hospital (in Italian). V. Nitti*, M. Parrella, and R. Cantarella. *Arch Tisiol*, July, 1959, 14: 647–661. (*Cl. Tisiologia, U. of Naples, Italy)

The susceptibility to streptomycin, isoniazid, and PAS of *M. tuberculosis* strains isolated from 120 patients at the time of their admission to hospital was studied in Löwenstein-Jensen medium. Only one case of apparently primary resistance was found among 35 patients never before treated with antimicrobials. Sixty-three per cent of the remaining 85 previously-treated cases had bacilli resistant either to one or more drugs. The appearance of resistance was dependent on the length of treatment, the clinical evolution of the process and, particularly, on whether the antimicrobials were given alone or in combination. It is concluded that most of the cases of resistance could be avoided by appropriate treatment. —I. Archetti

Modern Experience in Bacterial Meningitis. H. H. Hyland*. *Canad Med Ass J*, December 1, 1959, 81: 883–885. (*U. of Toronto, Canada)

Among 160 cases of bacterial or mycotic meningitis admitted to the Toronto General Hospital from 1947–1956, there were 48 cases of pneumococcal meningitis, 39 of tuberculous meningitis, 14 of staphylococcal meningitis, and 33 due to unknown organisms. The remaining cases were of varied etiology. The over-all recovery rate for tuberculous meningitis was 25 per cent but, prior to the introduction of isoniazid, the recovery rate was 5 per cent. Following the introduction of isoniazid, the recovery rate rose to 43 per cent. In 5 of the cases, tubercle bacilli could not be recovered from the spinal fluid. Intrathecal therapy is no longer used.—E. A. Riley

Outline of topics covered in the following issues of *The Medical Letter* (136 East 57 St., N. Y., N. Y.):

February 5, 1960: Hydroxyzine hydrochloride (Atarax®) and hydroxyzine pamoate (Vistaril®) in emotional disorders in children, cardiac arrhythmias, and allergic disorders. Calcium acetyl salicylate carbamide (Calurin®) as a substitute for plain aspirin. Appetite depressants in clinical obesity: dextro-amphetamine sulfate (Dexedrine®).

February 19, 1960: Trimethidinium methosulfate (Ostensin®), new ganglion-blocking agent in hypertension. A new parenteral tetracycline, N-pyrrolidinomethyl tetracycline (Syntetrin®). Antimicrobial prophylaxis for severe traumatic wounds.

March 4, 1960: Organic nitrates for the control of angina pectoris: nitroglycerin (sustained release tablets, Nitroglyn®); amyl or octyl nitrate; erythrityl tetranitrate (Cardilate® tablets); triethanolamine trinitrate biphosphate (Metamine®); pentaerythritol tetranitrate (Peritrate®); isosorbide dinitrate (Isordil®). Value of C.V.P. (U. S. Vitamin), a mixture of a water-soluble citrus bioflavonoid compound and ascorbic acid. Reassurance as well as medication in pinworm infestation.

March 18, 1960: Evaluation of cyclophosphamide (Cytoxan®). Vasopressor drugs for shock. Choline salicylate (Arthropan Lipuid®) as a substitute for aspirin in arthritis.

PULMONARY PHYSIOLOGY

Artificial Respiration and an Artificial Kidney for Severe Barbiturate Poisoning. G. E. Honey* and R. C. Jackson. *Brit Med J*, November 28, 1959, No. 5160: 1134–1137. (*United Oxford Hospitals, England)

A case of severe but probably accidental poisoning by butobarbitone is described in which mechanical artificial respiration and an infusion of L-noradrenaline were employed when spontaneous respiration failed and the blood pressure fell profoundly. The duration of coma was substantially reduced by using a Kolff twin-coil artificial kidney to dialyze butobarbitone from the blood, 1.51 gm. being extracted in 7 hours, compared to 0.63 gm. from the urine in 4½ days. The artificial kidney was transported 30 miles (48 km.) by road to the patient, who made a complete recovery (after authors' summary).—E. A. Riley

Levels of Blood Oxygen Saturation during Bronchoscopy in Children (in Japanese). Y. Yoshida* and H. Iwabuchi. *Jap J Clin Tuberc*, December, 1959, 18: 853–856. (*Nihon Med. College, Tokyo, Japan)

Levels of blood oxygen saturation were studied with the ear oximeter during bronchoscopy in 20 children on 23 occasions. There was a transient drop in the oxygen saturation level in most cases when the bronchoscope was passed through the larynx, but it returned to preoperative levels on further passage of the instrument. The degree of oxygen saturation was not dependent upon the location of the bronchoscope in the tracheobronchial lumen. Decreased oxygen saturation was more frequently seen in patients with bronchiectasis. A small amount of opium-scopolamine for older children and intravenous thiopental sodium (Pentothal® Sodium) for younger children was considered an adequate basal anesthetic in bronchoscopic examinations in order to ensure high levels of blood saturation with oxygen. Frequent suction of exudates and sputum was necessary for the prevention of hyposaturation of the blood with oxygen. It was recommended that the patients be given sufficient oxygen inhalation before bronchoscopy.—I. Tateno

The Pulmonary Diffusing Capacity in Bronchial Carcinoma. T. K. I. Larmi*. *Ann Chir Gynaec Fenn*, No. 3, 1959, 48: 341–355. (*Kirurginen Klinikka, Helsinki, Finland)

The diffusing capacity of the lungs was determined in 27 males with bronchial carcinoma by the single-breath method with carbon monoxide. The marked decrease in diffusing capacity in this series was due to the destruction of the pulmonary parenchyma, the decrease of the diffusing surface caused by emphysema and, possibly, to a chronic interstitial fibrosis of the lung. In 5 operated cases the diffusing capacity was decreased by an average of 20 per cent after a pneumonectomy.
—J. Haapanen

Cyanosis, Clubbing and Arterial Oxygen Unsaturation Associated with Laennec's Cirrhosis. T. Rodman*, J. K. Hurwitz, B. H. Pastor, and H. P. Close. *Amer J Med Sci*, November, 1959, 238: 534–541. (*V. A. Hosp., Philadelphia, Pa.)

Extensive pulmonary function studies were done on a 60-year-old man with the clinical syndrome of hepatic cirrhosis, cyanosis, and phalangeal clubbing. Severe arterial oxygen unsaturation was present, the result of a right-to-left shunt of approximately 40 per cent of the cardiac output. No anatomic lesion to account for the shunt was found clinically or by post mortem examination. Although the presence of multiple minute pulmonary arteriovenous fistulas has been suggested as the etiology of this syndrome, no such fistulas were demonstrated by plastic injection of one lung in this patient. It is suggested that portopulmonary venous communication by way of the mediastinal and bronchial network may be responsible for the shunt.—W. J. Steininger

Cardiopulmonary Insufficiency in Myotonic and Progressive Muscular Dystrophy. K. H. Kilburn*, J. T. Eagan, H. O. Seiker, and A. Heyman. *New Engl J Med*, November 26, 1959, 261: 1089–1096. (*V. A. Hosp., Durham, North Carolina)

Cardiopulmonary function was assessed in 17 patients with progressive muscular dystrophy, 9 of whom exhibited myotonia. Twelve patients had dyspnea or other respiratory symptoms. The total lung capacity was reduced in all of the patients, largely by loss of inspiratory reserve volume. The vital and maximal breathing capacities were impaired to a greater degree in the nonmyotonic patients. The patients with myotonia, on the other hand, showed a reduction in minute ventilation, carbon dioxide retention, and a decrease in ventilatory response to carbon dioxide inhalation. Pulmonary hypertension and a reduced arterial-blood oxygen saturation were also observed in some of the myotonic patients. Oxygen inhalation resulted in further reduction in respiratory minute volume and an increase in carbon dioxide tension in 3 of the patients with myotonia, changes that were not observed in those with nonmyotonic dystrophy. These observations indicate that the hypoventilation syndrome may appear in myotonic dystrophy, and is probably the result of weakness of the chest bellows, increased work of

breathing due to myotonia, and a disturbance in central respiratory mechanisms (Authors' summary).—M. J. SMALL

Obstructive Emphysema in Cigarette Smokers. A. L. FLICK* and R. R. PATON. *AMA Arch Intern Med*, October, 1959, 104: 518–526. (*V. A. Hosp., Seattle, Wash.)

Evidence indicating cigarette smoking as the likely cause of "diffuse hypertrophic idiopathic emphysema" is presented. Data were analyzed on the smoking habits and the maximal expiratory flow rates (MEF) of 222 male patients, MEF being selected as the best single screening test for emphysema. The data indicate that there is a spectrum of loss in the ease of expiration (measured by decreased MEF) which becomes apparent after some 30 years of smoking and which progresses with increased smoking. Nonsmoking men show minimal change in MEF to age 70. Many smokers show moderate to marked decline in MEF with increasing age, heavy smokers showing more loss in MEF than do light smokers. Smokers who leave short cigarette butts show more loss than those who leave longer butts. Habitual cough, habitual raising of sputum, and "chest colds" are more common in smokers than in nonsmokers. The pathogenesis of emphysema is reviewed and discussed, with emphasis on terminal bronchiolitis as the primary lesion.
—W. J. STEININGER

The Electrocardiographic Pentalogy of Pulmonary Emphysema. A Correlation of Roentgenographic Findings and Pulmonary Function Studies. R. H. WASSERBURGER*, J. R. KELLY, H. K. RASMUSSEN, and J. H. JUHL. *Cardiology*, November, 1959, 20: 831–841. (*V. A. Hosp., Madison, Wisconsin)

The clinical applicability of the routine electrocardiogram and the chest roentgenogram in assessing advanced pulmonary emphysema is shown by a correlative study on 150 adults with moderately severe and severe pulmonary emphysema diagnosed by pulmonary function studies. From these data, a fairly uniform electrocardiographic pattern has emerged titled "the electrocardiographic pentalogy of pulmonary emphysema" which, by itself, suggests the presence of an advanced emphysematous state. This pattern consists of: (*1*) prominent P-waves in leads II, III, and aV_F; (*2*) exaggerated T-a waves greater than 1 mm. in leads II, III, and aV_F; (*3*) vertical cardiac position; (*4*) marked clockwise rotation; and (*5*) tendency to generalized low voltage, particularly in the left precordial leads V_4 through V_7. The T-a–wave inscription and the precordial QRS complex alteration may be of such extreme degree that myocardial disease is suggested. Failure to appreciate these electrocardiographic changes as expressions of advanced emphysematous states may alter the therapeutic approach.
—W. J. STEININGER

Pulmonary Ventilation and Perfusion in Normal Subjects and in Patients with Emphysema. J. READ*. *Clin Sci*, 1959, 18: 465–473. (*Postgraduate Med. School of London, England)

Pulmonary ventilation—blood flow relationships were studied by continuous analysis of single expirates with a mass spectrometer in 34 normal subjects and in 19 patients with emphysema. In normal male subjects, ventilation, blood flow, and the ventilation-perfusion ratio became more uneven within the lungs with increasing age. Ventilation was uneven in all of the patients with emphysema; in 11, pulmonary blood flow was no more uneven than in normal subjects of the same age. In 8 patients, pulmonary blood flow was distinctly uneven, and partially or completely "compensated" uneven ventilation, so that there was relatively less inequality of ventilation-perfusion ratios within the lungs. The possible significance of this finding is discussed (after author's summary).—H. J. SIMON

The Measurement of Pulmonary Diffusing Capacity for Carbon Monoxide by a Rebreathing Method. B. M. LEWIS*, T.-H. LIN, F. E. NOE, and E. J. HAYFORD-WELSING. *J Clin Invest*, November, 1959, 38: 2073–2086. (*Wayne State U. College of Med., Detroit, Mich.)

A rebreathing method is described for the measurement of pulmonary diffusing capacity for carbon monoxide (DL_{CO}) using stable CO and continuous analysis. There is a high correlation between rebreathing and single-breath measurements of DL_{CO} in normal subjects and patients without airway obstruction. There is a less striking correlation between these two methods in patients with emphysema. The rebreathing method is rapid, simple, and gives a simultaneous measurement of DL_{CO} and residual volume.
—E. DUNNER

Reduction in Systemic Blood Oxygen as a Result of Procedures Affecting the Pulmonary Circulation in Patients with Chronic Pulmonary Disease. D. F. HALMAGYI* and J. E. COTES. *Clin Sci*, 1959, 18: 475–489. (*Llandough Hosp., Nr. Penarth, Glamorgan, Wales)

In a group of 6 patients who had epinephrine (Adrenalin®)-sensitive bronchospasm, small but significant reductions in arterial oxygen tension and saturation were found following the adminis-

tration of hyperduric epinephrine, as compared to physiologic saline, in a controlled experiment. Reductions in arterial oxygen saturation were also observed following the use of aminophylline in 8 patients who experienced relief of bronchospasm, but not in 5 others who did not. A reduction was observed following the use of nicotinic acid in 4 instances; in contrast, 4 subjects who received physiologic saline all showed small increases in saturation. Similar reductions are reported in the literature following the administration of cardiac glycosides, ganglionic blocking agents, chlorpromazine, and change of posture in patients with mitral stenosis and chronic pulmonary disease.

It is suggested that in patients with pulmonary hypertension these procedures may give rise to increased venous admixture either because of increased cardiac output or reduced pulmonary arteriolar resistance. In either event the arterial blood oxygen will be reduced. The extent of reduction may be affected by concurrent changes in ventilation minute volume (after authors' summary).—H. J. SIMON

Pulmonary Function Study Data after Plombage (in German). W. SCHULTZE-BRÜGGEMANN*. *Schweiz Z Tuberk*, No. 1, 1959, 16: 39–46. (*Sanatorium "Schildautal," Seesen a. Harz, Lautenthaler Strasse, Germany)

The ventilatory function of the lungs is less impaired after plombage than after thoracoplasty. This conclusion—corresponding with the observation of other authors—was reached following 4,000 pulmonary function studies in 425 patients. —Z. VIRÁGH

Nitrogen Clearance Rates of Right and Left Lungs in Different Positions. G. A. LILLINGTON*, W. S. FOWLER, R. D. MILLER, and H. F. HELMHOLZ, JR. *J Clin Invest*, November, 1959, 38: 2026–2034. (*Mayo Clinic, Rochester, Minn.)

Healthy subjects breathing oxygen had a slower rate of clearance of pulmonary nitrogen in the lateral decubitus position than when they were supine. A partial anatomic localization of the variously ventilated regions was attempted by measuring nitrogen clearance rates and the dead space of individual lungs of 5 normal men in the supine and both lateral decubitus positions by use of bronchospirometric catheters. Clearance curves of multiple exponential form were found, as Svanberg has noted, indicating the presence of uneven ventilation within individual lungs in all positions. In the supine position, the tidal volume, dead space, and functional residual capacity were greater for the right lung than for the left, but the clearance rates and relative magnitude of slowly and rapidly ventilated regions of both lungs were similar. In lateral decubitus positions, the ventilatory pattern of the dependent lung was similar to that of the same lung in the supine position. The superior lung had an increased functional residual capacity and a decreased tidal volume. The clearance rates of both rapidly- and slowly ventilated components of a lung were only half as great when it was in the superior position as they were in the same lung when the subject was supine. The slow clearance rates of the superior lung appear to be largely responsible for the retardation of nitrogen clearance from the total lungs observed in lateral decubitus positions. This represents a regional type of uneven alveolar ventilation. The variations between lungs with change in position show that relatively retarded alveolar ventilation is not always restricted to a particular anatomic location.—E. DUNNER

Changes in Hemodynamics Caused by Hypoxia in Pneumoconiotic Patients (in Italian). F. GOBBATO*, G. SEQUI, G. ZAGARESE, and A. BERRA. *Med Lavoro*, April, 1959, 50: 273–285. (*U. of Padua, Italy)

The variations in hemodynamics induced by hypoxia obtained by inhalation of gaseous mixtures low in oxygen (10 per cent of oxygen in nitrogen) for 16 to 22 minutes were studied in patients with pulmonary silicosis. According to the results, during hypoxia there was a marked increase of cardiac output, due more to an increase of cardiac rate than to the volume of systolic discharge. Hypoxia also caused arteriolar dilatation with consequent diminution of the peripheral resistance. Changes were more evident in persons with an abnormal respiratory function. By Levy's test, a latent coronary disease could be revealed in patients who had suspect symptomatology but a normal electrocardiogram at rest; the test could also be used to reveal the electrocardiographic signs of an early strain of the right heart in patients with chronic pulmonary disease. —I. ARCHETTI

Electroencephalographic Studies in Respiratory Failure. L. A. G. DAVIDSON* and J. M. JEFFERSON. *Brit Med J*, September 12, 1959, No. 5149: 396–400. (*U. of Birmingham, England)

Sixteen patients were studied during hospital admissions for acute or subacute respiratory failure, and the electroencephalograms (EEG), clinical signs and symptoms in the nervous system, and blood gas findings were compared. The EEG shows symmetric changes from intermittent theta activity to paroxysmal delta discharge of high amplitude, and the changes resemble those found in other types of confusional state, particu-

larly those of metabolic orgin. There is good correlation between the EEG abnormality and the clinical neurologic findings. There is also a correlation between increasing EEG abnormality and a falling pO_2 and rising pCO_2 and plasma CO_2 content when the patients are considered as groups, but not in individual cases. No relationship with arterial pH was observed. No other relationships were noted (after authors' summary). —E. A. Riley

Ventilatory Function and Its Relation to Pulmonary Circulatory Dynamics in Mitral Stenosis and Congenital Heart Disease (in Japanese). H. Saeki*. *Resp Circ*, December, 1959, 7: 1105–1124. (*Tokyo U., Japan)

The ventilatory function and pulmonary circulatory dynamics were examined in 34 cases of mitral stenosis and 38 cases of congenital heart disease, and the following results were obtained:

Mitral stenosis: (*1*) In mild cases with pulmonary capillary pressure below 20 mm. Hg. and pulmonary arterial pressure below 30 mm. Hg., lung volume and maximal breathing capacity showed normal values in the majority of cases. In patients with pulmonary capillary pressure above 20 mm. Hg. and pulmonary arterial pressure above 30 mm. Hg., decrease of vital capacity, increase of the ratio of residual volume to total lung capacity, and decrease of maximal breathing capacity were generally obvious. (*2*) There was a significant negative correlation between vital capacity and pulmonary capillary pressure or pulmonary arterial pressure ($r = -0.628$ and -0.642, respectively: $P < 0.01$). This relationship became greater when patients with extremely long or short duration of symptoms were excluded. (*3*) There was no significant correlation between the maximal breathing capacity and pulmonary capillary or pulmonary arterial pressure ($r = -0.502$ and -0.353, respectively: $P > 0.05$). (*4*) Air velocity index was 0.77–1.49, the average value being 1.11.

Congenital heart disease: (*1*) Congenital heart disease with left to right shunt: (*a*) Lung volume and maximal breathing capacity generally showed normal values in patients with distinct increase of pulmonary blood flow but without elevation of pulmonary arterial pressure. (*b*) Decreased vital capacity, increased ratio of residual volume to total lung capacity, and decreased maximal breathing capacity were observed in the majority of patients with marked increase of pulmonary blood flow and distinct elevation of pulmonary arterial pressure. (*c*) In a patient who showed marked elevation of pulmonary arterial pressure but no increase of pulmonary blood flow, and which was accompanied chiefly by right-to-left shunt, there was no distinct change in lung volume and maximal breathing capacity. (*2*) In pulmonary stenosis and Fallot's tetralogy there was no distinct change in lung volume and maximal breathing capacity. In some of the patients with cyanosis, there was a marked increase in minute ventilation.—I. Tateno

MICROBIOLOGY AND IMMUNOLOGY

Bacteriologic Diagnosis of Mycobacterial Infections in Man (in Roumanian). C. C. Oprescu* and T. Ieremia. *Microbiol Parazitol Epidemiol*, July–August, 1959, 4: 337–357. (*Str. Aristide Briand 7, Bucuresti, Roumania)

A long and detailed account of the subject. —Z. Virágh

The Antigenic Constituents of Some Mycobacteria (in Italian). G. Castelnuovo*, A. Gaudiano, M. Morellini, G. Penso, and M. Polizzi-Sciarrone. *Ann Ist Forlanini*, No. 1, 1959, 19: 40–57. (*Ist. Superior Sanità, Rome, Italy)

The antigenic structure of several species of mycobacteria, some of which were pathogenic and some nonpathogenic, was studied by the agar double-diffusion method and by immuno-electrophoresis. The various antigens were prepared by mechanical disintegration of the bacteria at low temperature, following Zampieri's technique; the serum was obtained from rabbits. It was possible to detect a considerable number of antigens in the different species. According to the results of the reaction of identity and to the electrophoretic mobility, all of these mycobacteria have at least one common antigen, while some species have more than one. Tubercle bacilli presented the higher number of antigens: it appears that a slight antigenic difference exists among the human, bovine, and BCG strains.

The antigenic similarities between the various species and the group of tuberculous mycobacteria are analyzed, and the possibility of a certain correspondence between virulence and the presence of some antigens is discussed.—I. Archetti

Are There Human Pulmonary Diseases Due to Unclassified (Atypical) Mycobacteria Nonpathogenic for Animals? (in French). J. Viallier*, G. Despierres, and R. M. Cayré. *Presse Med*, August 22, 1959, 39: 1508–1510. (*Lyon, France)

This is the case report of a 37-year-old man who suffered from silicosis with a pseudotuberculous course. The chest roentgenogram showed bilateral diffuse nodular lesions with a necrotic density in the right apex. The patient eventually died of a pleuropulmonary perforation followed by pyopneumothorax. Unclassified (atypical) chromo-

genous mycobacteria were cultured repeatedly from the sputum and the pleural fluid. These organisms were nonpathogenic for test animals. Agglutination tests showed similarity to reactions obtained sometimes in the blood serum of tuberculous patients. The question was raised—but not answered—whether such bacilli, usually nonpathogenic, could become pathogenic in the presence of a coexisting lung disease such as silicosis.—E. LYON

Current Concepts of Diagnostic Serology and Skin Hypersensitivity in the Mycoses. S. SALVIN*. *Amer J Med*, July, 1959, 27: 97–114. (*National Institutes of Health, Rocky Mountain Lab., Hamilton, Montana)

The accompanying table summarizes the tests and the antigens employed therein. Some of these tests, such as the complement-fixation tests for histoplasmosis and coccidioidomycosis, have been employed extensively and are recommended for use as aids in diagnosis and prognosis. Others, such as the skin tests for cryptococcosis and actinomycosis, require further study before their true value can be ascertained.—T. H. NOEHREN

TABLE 1
IMMUNOLOGIC TESTS FOR THE DIAGNOSIS OF SIX MYCOTIC DISEASES

Disease	Agglutination	Test for Circulating Antibody		Skin Test
		Precipitation	Complement Fixation	
Histoplasmosis	+ (Latex-histoplasmin)	+ (Yeast phase filtrate or histoplasmin)	+ (Whole cells of yeast phase and histoplasmin)	+ (Histoplasmin)
Cryptococcosis	±	±	±	±
Coccidioidomycosis	−	+ (Coccidioidin)	+ (Coccidioidin)	+ (Coccidioidin)
Candidiasis	+ (Cells of yeast phase)	±	±	+
North American blastomycosis	−	±	+ (Whole cells of yeast phase)	+ (Blastomycin)
Actinomycosis	±	±	±	±

+ = Tests of value in diagnosis in man.
± = Tests have been reported but are not sufficiently advanced for use in routine diagnosis in man.
− = No satisfactory test.
( ) = Recommended antigen for test.

Bacteriologic Diagnosis of Tuberculosis by Fluorescent Microscopy (in Spanish). J. URDIALES CAMPOS*. *Enferm Torax*, No. 29, 1959: 83–111. (*Sanatorio de Viana, Valladolid, Spain)

Various specimens from 200 patients (sputum, bronchial washing, pleural effusion, pus obtained from lymph nodes) were tested for acid-fast bacilli by the simple Ziehl-Neelsen method, fluorescent microscopy, culture, and guinea pig inoculation. The fluorescent microscopic method gives better results than the Ziehl-Neelsen procedure, but it is inferior to the cultures. The best result was obtained from animal inoculation. —Z. VIRÁGH

Use of Fluorescent Microscopy in Laboratory Diagnosis of Tuberculosis (in Russian). M. E. KROL*, N. M. IVANOVA, and N. N. KATCHAOUNOVA. *Probl Tuberk*, No. 7, 1959, 84–89. (*Leningrad Institute of Tuberculosis, U.S.S.R.)

More than 5,000 examinations of 1,697 pathologic specimens from 826 patients were examined by fluorescent microscopy. Of these, 752 were sputum specimens, 560 bronchial washings, 76 specimens of tuberculous pus, 228 serous exudates, and 81 others. Two hundred of the 826 patients were known to have positive sputum. Tubercle bacilli were found in 435 patients. This method of examination increased the percentage of positive bacteriologic findings by 13 to 18 per cent. The method is quick and simple, and the frequency of positive results is almost equal to that obtained by culture.—W. A. ZAVOD

Peknice's Niacin Test as Modified by Bönicke and Lisboa (in German). E. GRABENDÖRFER-QUINCKE*. *Beitr Klin Tuberk*, April, 1959, 120: 150–156. (*Köln Poll, Siegburgerstrasse 358a, West Germany)

In employing this method, the mycobacteria are cultured in modified Lockemann's solution and tested by the Peknice reaction that uses sodium cyanide in place of the cyanogen bromide of Konno's test. Of the 732 strains of common tubercle bacilli examined, 562 were eugonic, 99 dysgonic, and 30 were moderately dysgonic. In addition, the test was used on several photochromogenic, unclassified ("atypical"), avian, BCG, and suspected-BCG strains. The specimens were obtained from bronchial secretions and extrapulmonary sources. All of the BCG and the moderately dysgonic strains, as well as 95 per cent of the eugonic strains, produced niacin. None of the avian, photochromogenic, and other unclassified mycobacteria were positive. The isoniazid-resistant strains behaved the same as the susceptible ones.

Peknice's method for testing the niacin-pro-

ducing properties of mycobacteria is equal in value to Konno's, but the testing procedure is simpler since the reagents are not poisonous and, thus, easier to use.—Z. Virágh

Simplified Niacin Test (in Spanish). J. M. Gutiérrez-Vázquez*. *Rev Lat Amer Microbiol*, December, 1958, 1: 286–296. (*Escuela Nacional de Ciencias Biologicas, Mexico 17, D. F.)

A modification in technique of Konno's niacin test for differentiation of human mycobacteria from other types is described. This test is carried out with sterilized aqueous solution of the growth on Löwenstein-Jensen medium of the mycobacteria to be tested. To achieve a better color reaction, an aromatic amine—e.g., *o*-tolidine—is employed before adding the usual cyanogen bromide to it; to reduce their amount, only drops of the reagents are used on an excavated spot-test porcelain plate. The color developed varies from blue (negative) to coral red (positive); only human mycobacteria respond with red.
—Z. Virágh

EXPERIMENTAL PATHOLOGY

Therapy of Experimental Pulmonary Edema in the Dog with Special Reference to Burns of the Respiratory Tract. D. M. Aviado*. *Circulat Res*, November, 1959, 7: 1018–1030. (*U. of Pennsylvania Schools of Med., Philadelphia)

The results of analysis of edema fluid and excised lungs of dogs following steam inhalation or intravenous injection of alloxan show that there is leakage of protein-rich fluid into the lung tissue and ultimately into the respiratory passages. This leakage cannot be explained by the reduction in oxygen uptake as observed in lung tissue slices. The survival time of anesthetized dogs suffering from thermal edema can be prolonged by inhalation of oxygen and by administration of antihistamine drugs and pulmonary vasodilators.
—W. J. Steininger

Nickel Poisoning. F. Sunderman*, A. Donnelly, B. West, and J. Kincaid. *AMA Arch Industr Health*, July, 1959, 20: 36–41. (*Jefferson Med. College, Philadelphia, Penn.)

Pulmonary cancers have been induced in rats subjected to a heavy single exposure to nickel carbonyl, as well as in rats subjected to repeated sublethal exposures for a period of a year. The cancers were not observed until two years or more after the initial exposure. These studies have shown that the inhalation of nickel carbonyl can cause pulmonary cancer in rats (Authors' summary).—T. H. Noehren

Immunologic Study of Serum Proteins Adsorbed on Crystalline Silica (in Italian). B. Pernis*, E. Clerici, and I. Ghezzi. *Med Lavoro*, June–July, 1959, 50: 405–411. (U. of Milan, Italy)

In order to test the hypothesis that immune response in silicosis is due to the capacity of crystalline silica to adsorb serum proteins and to transform them subsequently into auto-antigens, Ouchterlony's gel-diffusion precipitation method was employed. It was shown that quartz-adsorbed gamma globulins maintain their antigenic specificity. It is thought that these results do not support the point of view that immunologic reactions in silicosis are due to antibody production against auto-antigens formed by denaturated serum proteins altered by quartz absorption.
—I. Archetti

Effect of Chromium on Incidence of Lung Tumors in Mice and Rats. A. Baetjer*, J. Lowney, H. Steffee, and V. Budacz. *AMA Arch Industr Health*, August, 1959, 20: 124–135. (*Johns Hopkins U., Baltimore, Md.)

The exposure of mice and rats to a mixed chromate dust in a dust chamber did not lead to the development of bronchogenic carcinoma or to an increase in the incidence of benign tumors of the lungs, although spontaneous tumors began to appear at an earlier age in the dust-exposed mice. The intratracheal and intrapleural injection of this material also yielded negative results. Neither the intratracheal nor the intravenous injection of basic potassium zinc chromate or barium chromate caused bronchogenic carcinoma or an increase in pulmonary adenomas in mice. The intratracheal injection of zinc chromate produced an epithelization of the alveoli, and both the intratracheal and intravenous injection of the zinc chromate led to an earlier appearance of adenomas (Authors' summary).—T. H. Noehren

PUBLIC HEALTH AND EPIDEMIOLOGY

Epidemiological Basis of Tuberculosis Eradication in an Advanced Country. E. Groth-Petersen*, J. Knudsen, and E. Wilbek. *Bull WHO*, No. 1, 1959, 21: 5–45. (*Danish Tuberculosis Index, Copenhagen, Denmark)

In the first section, an outline is given of the changing tuberculosis situation in Denmark during the past several decades. The prevalence of tuberculous infection in children and the prevalence of bacillary cases of pulmonary tuberculosis in adults are suggested as the most valuable indices of eradication.

In the second section, the nation-wide mass

campaign of 1950–1952 is described. It consisted of a tuberculin test with 10 TU and a photofluorogram of all persons 15 years and older. All persons with negative tuberculin reactions were offered BCG vaccine. Details are given as to the results of the tuberculin tests and the roentgenographic examinations, and of the relation between vaccination status, tuberculin reaction, and roentgenographic findings.

In the third section, the results of the findings obtained during the first 4-year follow-up period are discussed. Seventy-five per cent of the new cases of pulmonary tuberculosis appeared among the unvaccinated tuberculin-positive population. Groups with definite high risk as to the development of active tuberculosis are positive reactors with definite lung lesions of probable tuberculous origin which showed no change during the initial 6-month follow-up period, and persons with a tuberculin reaction 12- to 23-mm. in size. The incidence of tuberculosis in the follow-up period is much lower in the older than in the younger population, and the association between the size of the tuberculin reaction and morbidity is much less pronounced. Within each of the risk groups, the incidence rates show a steady downward trend with increasing age. Persons in the older age groups with normal findings on a single survey chest film need not be called back for examination year after year. But persons of any age with suspicious lesions and young people with large tuberculin reactions should be followed systematically. —H. Abeles

Some Observations on the More Recent Tuberculin Surveys in Italy (in Italian). M. Barberio*, D. Oricchio, and V. Rustichelli. *Lotta Tuberc*, June, 1959, 29: 805–822. (*Istituto Forlanini, U. of Rome, Italy)

Some of the data obtained during various surveys employing the percutaneous tuberculin test in the Italian population since 1952 is briefly discussed. The 326,033 cases observed involved school and pre-school children, military personnel, young people chosen at random in the normal population, and persons living in contact with tuberculous patients. The mean values of the tuberculin index were as follows: 16.75 per cent among 277,829 subjects among the school and preschool children less than 14 years old; 29.14 per cent in the young soldiers; 16.12 per cent in the randomly chosen young people less than 14 years old; and 70 to 80 per cent among the children of tuberculous or ex-tuberculous patients. The conclusion of this study favors prophylactic chemotherapy among these population groups. —I. Archetti

Health Protection in Beryllium Facilities. A. Breslin* and W. Harris. *AMA Arch Industr Health*, June, 1959, 19: 596–648. (*U. S. Atomic Energy Commission, New York Operations Office, New York)

Concurrence is unanimous among investigators that beryllium per se is the causative agent of the occupational illness which is unique to the industry. On the other hand, it has been clearly demonstrated that the application of suitable engineering control measures can make the handling of beryllium and its compounds no more hazardous than that of other more common toxic industrial materials such as mercury and lead. Experience gained over a period of about ten years in the operation of beryllium facilities under contract to the Atomic Energy Commission has resulted in practical, proven control measures that can serve as guides in the engineering design and safe operation of new or modified installations. The facilities represent a wide spectrum of types of operation from small laboratories to full-scale production plants with processing rates from grams to tons per month. The purpose of this report is to summarize the information gathered during the design and operation of these facilities so that it may be used in providing for the safe handling of beryllium materials.—T. H. Noehren

The Distribution and Retention of Chromium in Men and Animals. A. Baetjer*, C. Damron, and V. Budacz. *AMA Arch Industr Health*, August, 1959, 20: 136–150. (*Johns Hopkins U., Baltimore, Md.)

Epidemiologic evidence has demonstrated that men employed in the chromate chemical manufacturing industry have a higher rate of bronchogenic carcinoma than do comparable control groups. The experiments described in this paper were undertaken to investigate the role of chromium chemicals in bronchogenic carcinoma. The following results were obtained: Analysis of lung tissue from men who had worked in a chromate chemical–producing plant indicated that the presence or absence of lung cancer was not directly related to the concentration of soluble or insoluble chromium (chromite) in the lung tissue. Soluble as well as insoluble chromium was found in the lung tissue of men who developed cancer many years after their exposure ceased. The soluble chromium found in the lungs of men at autopsy was firmly bound by cellular tissue and was insoluble in saline, acetone, and dilute base, but readily soluble in concentrated acid. The relative importance of hexavalent and trivalent chromium as factors in the production of bronchogenic carcinoma is discussed.—T. H. Noehren

Emergency Respiratory Protection Against Radiological and Biological Aerosols. H. GUYTON*, H. DECKER, and G. ANTON. *AMA Arch Industr Health*, August, 1959, 20: 91–95. (*Biological Warfare Lab., Fort Detrick, Md.)

Eighteen variations of 8 household and personal items were evaluated in an experiment with human subjects to determine the potential utilization of the objects as emergency respiratory protection expedients in the event of a radiologic or biologic attack. The items were: (*1*) a man's cotton handkerchief, (*2*) a woman's cotton handkerchief, (*3*) cotton dress material, (*4*) a muslin bed sheet, (*5*) a man's cotton shirt, (*6*) a rayon slip, (*7*) a turkish bath towel, and (*8*) toilet paper. Five variations of these items have a filtration efficiency greater than 85 per cent. They are: a man's cotton handkerchief folded to a thickness of 16 layers, folded to a thickness of 8 layers, or placed over the mouth in a crumpled state; a commercially available toilet paper, when used in a thickness of 3 sheets; a bath towel, when folded in 2 layers. However, the high resistance of the handkerchief when folded to give 16 layers and when crumpled limits the usage of these 2 variations to short intervals of time.

In order to determine whether wet items were superior to dry items, 5 of the cloth materials were evaluated after being saturated with water and subsequently hand-wrung. The most efficient of these items was found to be the bath towel (70.2 per cent). This result is less than that obtained for 7 dry items. Four of the 5 wet items exhibited extremely high resistance to breathing. Therefore, the use of wet items as respiratory protective expedients appears impractical.—T. H. NOEHREN

Synergistic Effects of Aerosols. II. Effects on Rate of Clearance from the Lung. C. LABELLE* and H. BRIEGER. *AMA Arch Industr Health*, August, 1959, 20: 100–105. (*Jefferson Med. College, Philadelphia, Penn.)

The rates at which inhaled foreign particulates are eliminated from the lung after exposure differ widely: the extreme values for the half-life in the lung are 1 hour for metallic iron particles and 5¼ months for beryllium sulfate. A part but not all of this variation is associated with differences in particle size: large particles, in general, are expelled more rapidly than small particles. Particulates reaching the lung in very small amounts may be retained in the lung for unexpectedly long periods. This would likely be true of the particulates present in the general atmosphere as a result of smog or fall-out. Predictions as to the effect of smog or fall-out which are based on analogies with industrial experience may be misleading, since industrial dust levels are commonly adequate to stimulate elimination by phagocyte production. The simultaneous administration of inert dust can accelerate the expulsion of biologically active particles from the lung when the latter are present in very small amounts. The greater lung burden increases the release of phagocytes.

Accidental exposures to highly noxious particulates (e.g., radium sulfate aerosol) have occurred in the past and doubtless will occur in the future. Decontamination procedures which are more or less effective have been developed both for internal and external depots with the exception of the lung itself, in which the hazard of such deposition is perhaps the greatest. It is at least conceivable that the synergistic stimulation of phagocytes by inert particles might permit therapeutic decontamination of the lung (after authors' summary).—T. H. NOEHREN

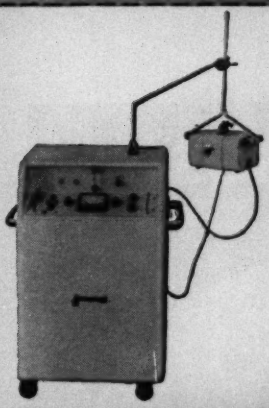
Oxygen Consumption of Male Indian Jute Workers. A. GUPTA*, B. FERRIS, JR., and M. RAO. *AMA Arch Industr Health*, August, 1959, 20: 96–99. (*Harvard School of Public Health, Boston, Mass.)

Data are presented on the oxygen consumption of male Indian jute workers during their work. The results are discussed in the light of the variability of work in industry.—T. H. NOEHREN

Tuberculosis in Indian Immigrants. J. T. NICOL ROE*. *Tubercle*, October, 1959, 40: 387–388. (*Chest Clinic, Uxbridge, Middlesex, England)

Attention is drawn to the apparently high incidence of active tuberculosis in immigrant Indians in Southall, England. Analysis has suggested that more than half appear to have acquired the infection in England. The fact that the majority are Sikhs from rural areas who may often be tuberculin negative on arrival affords a possible explanation. This appeared to be confirmed by the large proportion with primary disease, pleural effusion, and miliary tuberculosis of the lungs (15 of 35). Of 44 Indians Mantoux tested (OT 1 in 1,000), 29 were tuberculin negative and only 15 tuberculin positive.

All Indians should be roentgenographed as soon after arrival as possible. They could be tuberculin-tested at the same time, and those proving tuberculin negative should be offered BCG vaccination. Their standard of living is generally lower than that of the British people of comparable economic status. Efforts are therefore necessary to ensure that this is improved, particularly with regard to the overcrowded conditions in which they live. —M. J. SMALL



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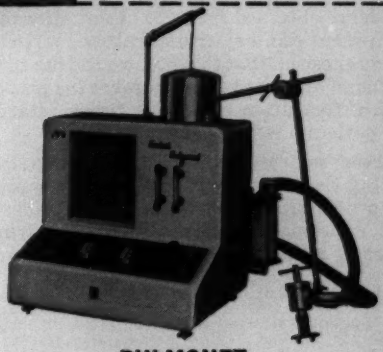

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1. Cohen, R. V., Lambert, R. L., and Molthan, L.: The Use of POTABA in Sarcoidosis; Annual Meeting American Trudeau Society, Eastern Section, Boston, Mass. (1958).
2. Zarafonetis, C. J. D., and Horrax, T. M.: Treatment of Peyronie's Disease with POTABA, Journal of Urology, 81: 770-772 (June 1959).

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